

Microbiology

Handwritten Note

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Name: _____

Subject: _____ Microbiology _____



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BACTERIAL VIRULENCE FACTORS

17/2/18

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Responsible for pathogenesis

Vaccine produced is also by virulence factors

Q. Smooth → virulent strain → so there are used for vaccine
rough → avirulent strain

Q. All of the following can be used as vaccine to prevent E. coli diarrhoea except

a) CS2

b) K88

c) CFA

d) P₁

All are fimbriae intestinal epithelial cells

a, b, c show adhesion to intestinal epithelial cells.
d shows adhesion to weighted epithelial cells.

FIBRILLAE (PILI)

- They are Glycoprotein

- Antigenic

- agglutinate RBC

- Nomenclature is based on the RBC agglutinated by RBC.

Function:-

1) Adhesion (Gram -ve)

In Gram +ve → adhesion is due to Teichoic Acid

2) Conjugation.

Mechanism of transfer of genetic material

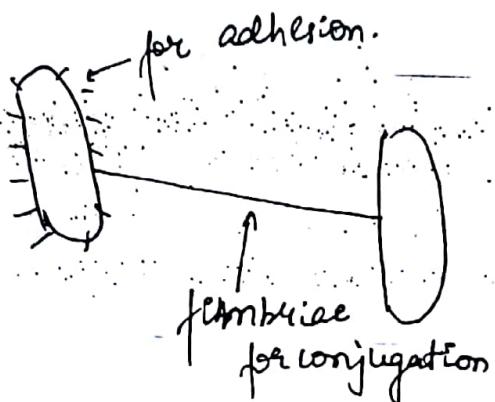
They are Plasmid encoded

F(-) bacteria can't initiate conjugation

Enterococcus conjugates to out fimbriae.

Plasmid encoded fimbriae

- Mannose resistant fimbriae
- CFA (Colonizing Factor antigen) [ETEC]
- Type 1 fimbriae



FLAGELLA

- Protein

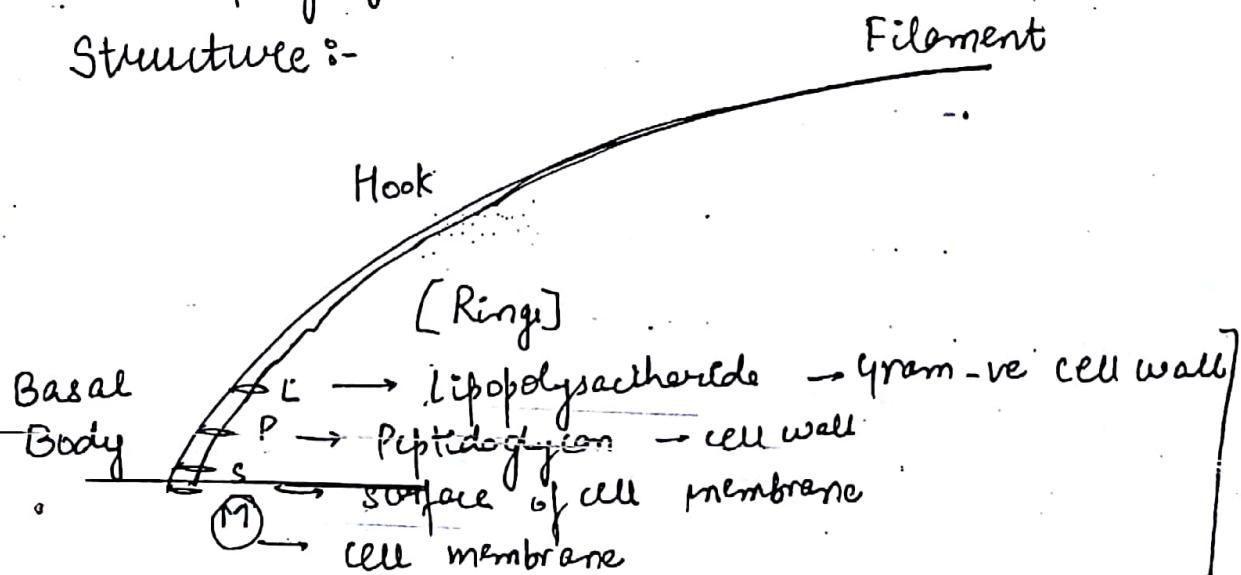
- antigenic 'H' Ag
- thin $< 0.02 \mu\text{m}$

Function :-

1) Motility - due to rotation of M Ring

2) Antiphagocytosis

Structure :-



In Gram -ve \rightarrow all flagella are found 5

In Gram +ve \rightarrow only S.M. flagella

↓
only cell membrane attachment

Motility helps in identification of bacteria

Flagella

Resolution Power

naked eye \rightarrow $200 \mu\text{m}$

Light microscope $\rightarrow \frac{200 \mu\text{m}}{1000} = 0.2 \text{ mm}$

$= 200 \text{ nm}$

Electron microscope $= \frac{200 \text{ nm}}{1000} = 0.2 \text{ nm}$

Flagella can't be seen by light microscope.

Demonstration of Flagella/Motility :-

\rightarrow Electron microscope

\rightarrow Ryu stain

silver based stain.

not sensitive

\rightarrow Serology.
 H antigen.

\rightarrow Hanging Drop (for vibrio only)

\rightarrow Dark ground microscope (spirochaetes)

But
Method Growth in semisolid agar (Motility medium)
 \downarrow
0.2 to 0.5% agar

Pennichorous Flagella -

6

flagella all round
Enterobacteriaceae

Lophotrichous Flagella -

Lophare camp me Halla

left of flagella at one end.

Campylobacter
Helicobacter

Single polar

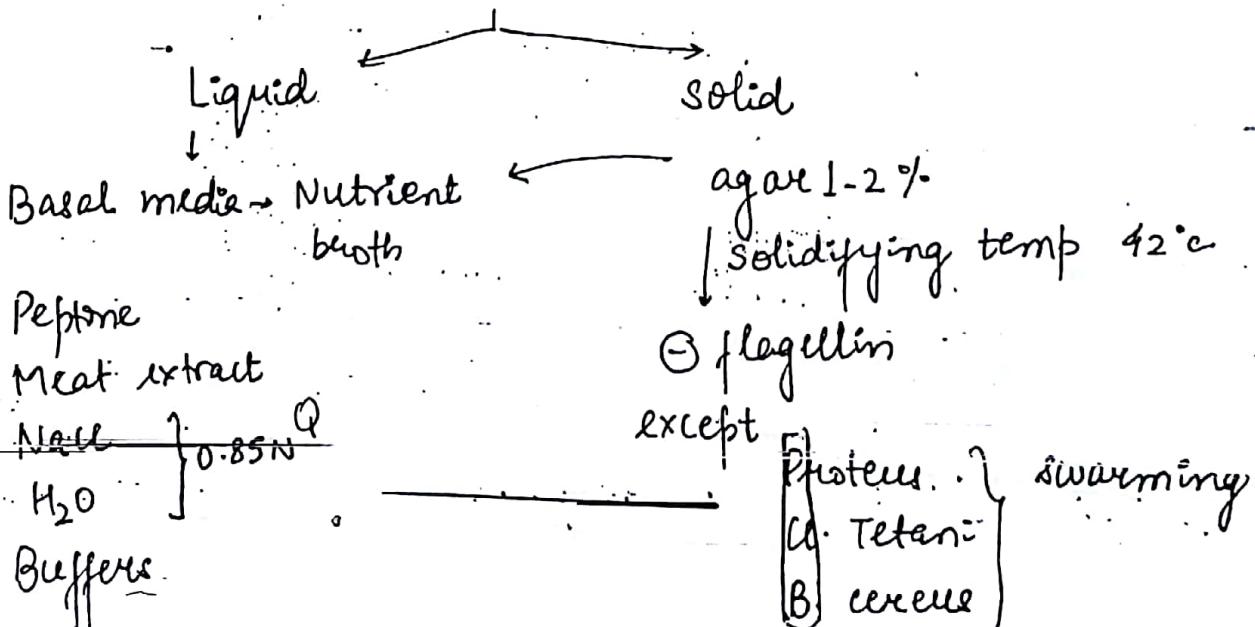
Vibrio

pseudomonas

Swarming can be inhibited by all except --

- a) 6% agar
- b) phenol agar
- c) blood agar
- d) Mc Conkey agar

Culture Media



Inhibition of swimming → 6% agar
 Phenol
 Boric Acid
 Bile salts (not in McConkey)

Mraigin's tube contain semisolid media but ~~is not~~ is not used for motility.

But in case of solid, semisolid media, it can be preferred for motility.

CELL WALL

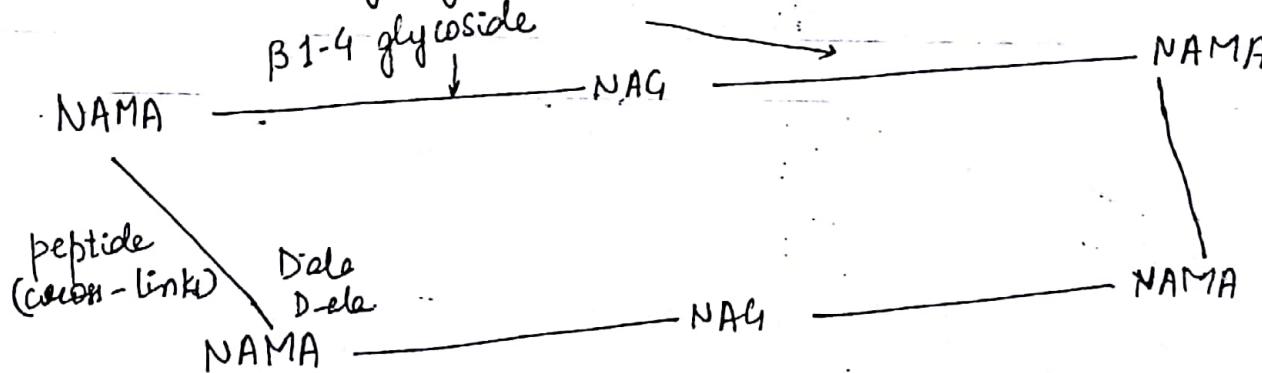
Peptidoglycan

Structure --

NAMA → N-acetyl muramic acid

NAG → N-acetyl glucosamine

$\beta 1-4$ glycoside



COO⁻ terminal in NAMA has unsaturated end, so form bonds while NAG doesn't have this.

Synthesis:-

Peptidoglycan is synthesised by **PBP** (penicillin Binding protein)

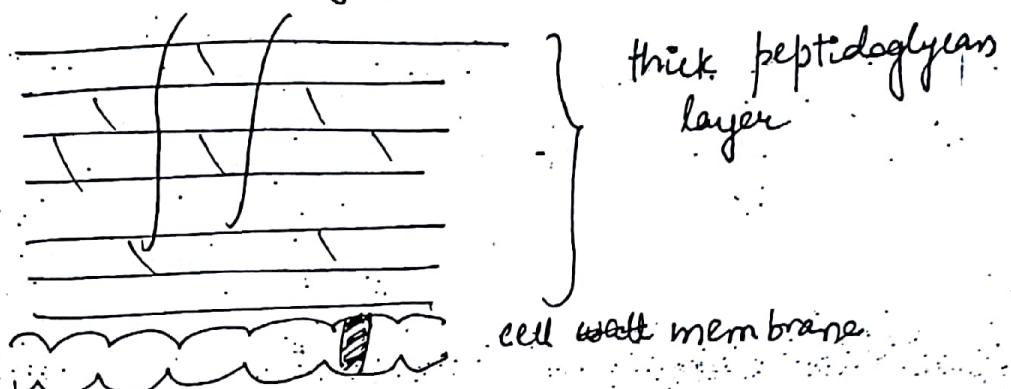
located in cell membrane

cross-linking (4th Phase. → last phase)

Types of cell wall

Gram +ve

Glycocalyx



Gram -ve

outer membrane
↓
Pores

Periplasmic
space

Region-I (outer polysaccharide)

Region-II (core ")

Region-III (lipid A) → most toxic

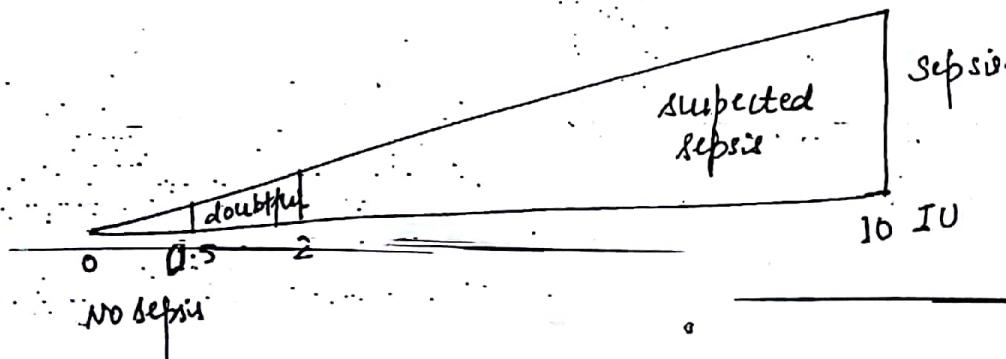
LPS

[endotoxin]

thin peptidoglycan

Lipid A → binds to Toll like Receptor 4 on macrophages.
(most toxic)

Best marker of Sepsis = **Pro calcitonin**



LPS → Hydrophobic

Pores help in passive diffusion of hydrophilic agents

Region I (outer polysaccharide) is antigenic

It is called as O antigen

O Antigen

→ useful for diagnosis (immunogen)

→ Serological ⇒ Serotyping (variability of O antigen)

↓
epidemiology Q.

Cholera endotoxin doesn't cause virulence

Acid Fast Cell Wall :-

Gram +ve cell wall = Mycolic acid

Function:-

Rigidity (cell wall)

CAPSULE

- Polysaccharide.

except B. anthracis → made up of polypeptide
(D-Glutamic acid) Q.

- Polysaccharides are not stained. ~~But~~

But capsule of B. anthracis can be stained by

Polychromatic Methylene Blue (Mc Fadyean Reaction)

Function:

Antiphagocytosis Q.

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Demonstration of capsule

1) M/I \Rightarrow Negative stain \rightarrow India Ink
or
Nigrosin

2) Serology a) Quellung Rx
swelling of capsule using capsular Ab.
 \downarrow
Microscope

b) Capsular Ag detection by Latex agglutination



Reverse passive agglutination



when Ag is
detected, then
it is called
reverse passive.

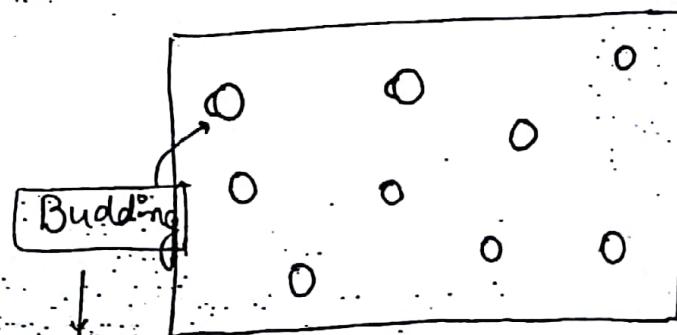


when particles are
used for Ab detecⁿ
e.g. latex
RBCs

Q: A pt. presented = headache, projectile vomiting along w/
altered sensorium Image of India ink

Δ

- (a) cryptococcus
- (b) pneumococcus
- (c) Haemoplasma
- (d) coccidioides



seen in Cryptococcus

pneumococcus shows Lancelet shaped.

Diphlococci

Gram -ve → Neisseria

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Gram +ve → Pneumococci

Neisseria (Intracellular Diphlococci)

Meningococci

Capsulated

Gonococci

Uncapsulated

Kidney shaped

Serological assay on microscope slides are not done except VDRL

Quellung Reac.

↓
Non-specific test

Polyvalent antipolysaccharide serum

Capsulated Organisms

Yes the PM cooks very fine chicken to keep his BBB healthy

- Yersinia

- Pneumococcus

- Meningococcus

- Cryptococcus

- Vibrio parahaemolyticus

Vibrio cholerae O₁₃₉ O

- fecal streaks of E. Coli, staph, Strep, pseudomonas

↓
Nosocomial (HAT)

- Clostridium perfringens & Butyricum

Klebsielle

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- *B. anthracis*

- *Bordetella*

- *Bacillus*

- *Burkholderia pseudomallei*

- *Haemophilus influenzae*

* Histoplasma capsulatum

✓ Non-capsulated.

✓ intracellular yeast & appear as capsulated

ENZYMES & TOXINS

Secreted by Secretion System.

→ Seven Pathways - type I to VII

Type I & IV → Both in Gram +ve & Gram -ve

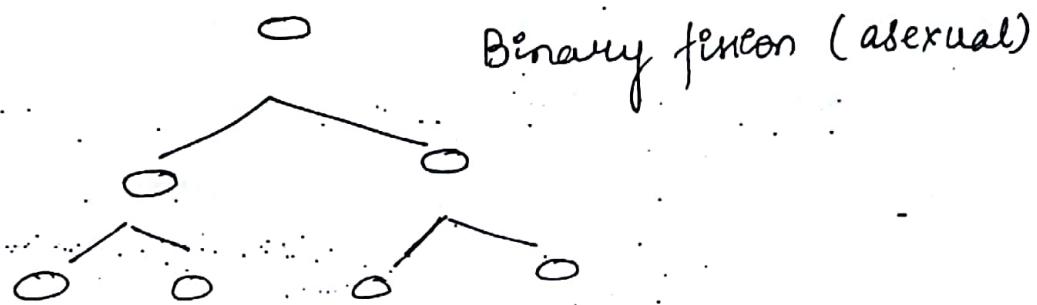
Type II, III, V, VI → only in Gram -ve

Type VII → M. TB.

→ Proteins secreted by Type I, III, & VI pathways - transverse inner membrane & outer membrane in one step
(see independent - Do not involve amino terminal processing of secreted protein)

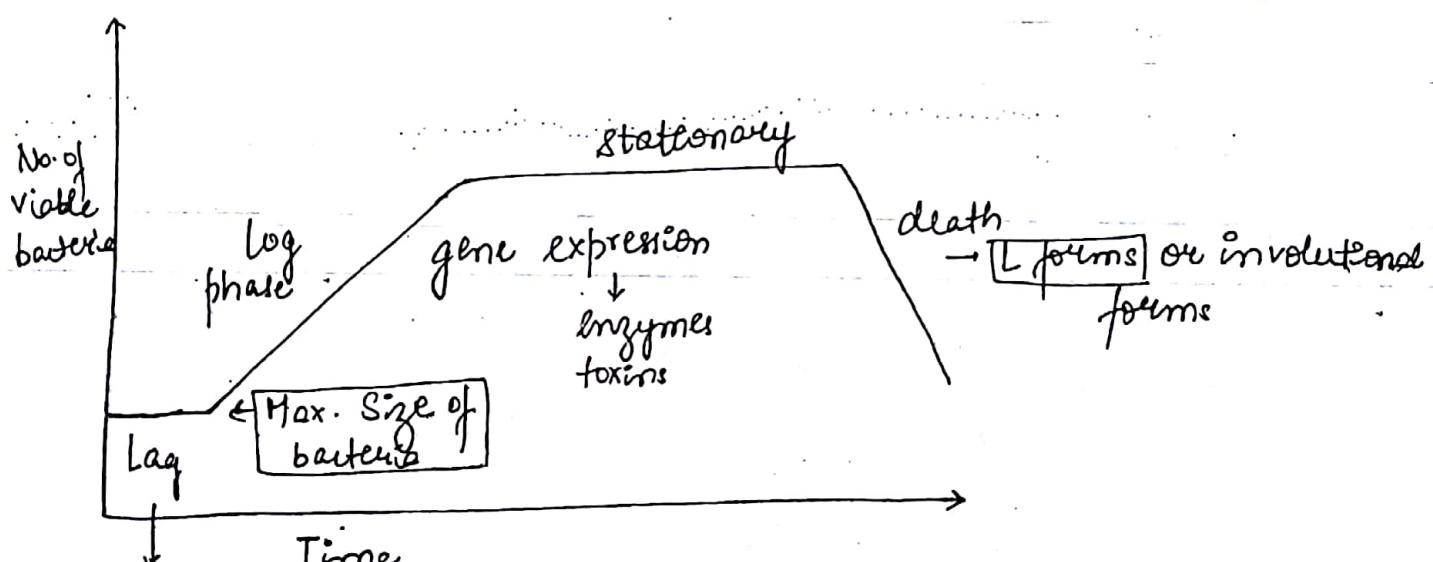
Growth Curve

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Doubling Time is called Generation Time
Max generation Time seen in M. Lepree - 13-14 days

M. Tb → 16 hrs
 other bacteria → 20 min. H/C



preparatory phase

↓
enzymes are released

[Metabolic Phase]

Anti microbial susceptibility is done by Phenotype Method
 ↓
in log phase

→ **Stationary phase** is due to toxic metabolic products
→ **bacterial colony form** → exhaustion of nutrients.
on solid medium

* **Sporulation** is seen in **stationary phase**. & this is for survival.

They are highly resistant bcoz they have
Ca dipicolinic acid synthetase

↓ absorption of water



↑ Resistance

A - **0.5% H_2SO_4** → Zn stain

L forms -

No cell wall

Mycoplasma

- **Spheroplast** → Gram -ve organisms
incomplete destruction of cell wall

↓
Reversible

- **Protoplast** → Gram +ve organism
complete destruction of cell wall



Irreversible

- L-forms are virulent

Q. A 25 year old lady presents w/ frequency & dysuria w/ from \oplus bacilli in urine?

After 2 weeks of penicillin t/t she comes back w/out resolution of her complaints?

w/ gram -ve cocci.

a) β -lactamase producing strain

b) Gonococci

c) Sphaeroplast

d) Protoplast

Rx- Discontinue Rx for 24-48 hr. ~~+ tell the doctor~~

Start the drug w/ proper dosage

BACTERIAL GENETICS

Prokaryotes

↳ No membrane bound organelle.

(no nu., mitochondria; no ER, golgi complex)

↓
Respiratory func → miosome.

(invagination of cell membrane)

No histone proteins.

↓
Packaging of DNA → supercoiling

Genes are located in chromosome

Plasmid

Transposon.

Chromosome • Plasmid → ds DNA

circular

self replicating

↓
DNA polymerase.

Plasmid are extra-chromosomal.

↓
No metabolic func

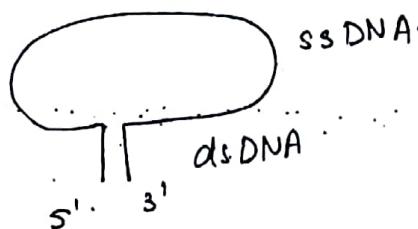
Mobile

Mobile Genetic Transposon Elements

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① Transposon

oligonucleotide stranded at complementary ends.



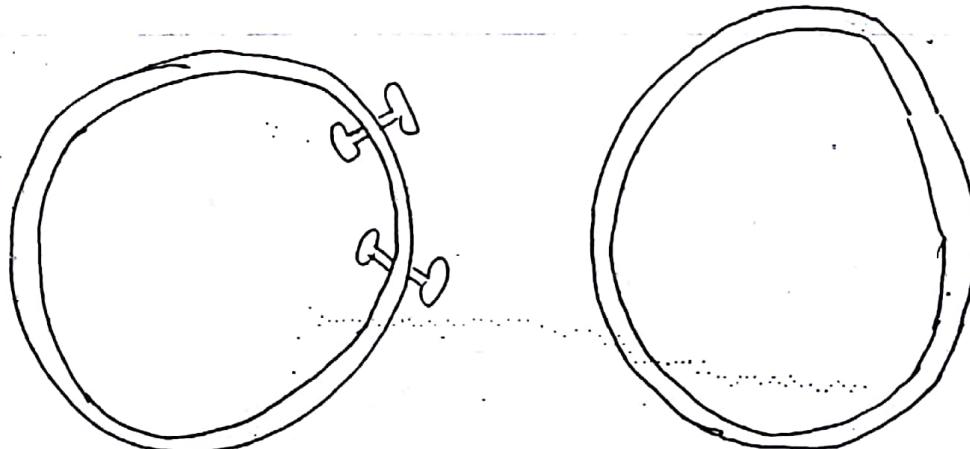
carry gene → impart new character

Transposons move in cut + paste manner bind to tRNA

↓ 'Jumping Gene'

Insertion is without complementation.

↓
insert directly between nucleotides → Repeat sequence formation



Plasmid mediated drug resistance is due to insertion of Transposons.

② Insertion Sequence (IS)

similar to transposon (smaller)

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No gene (phenotypic silence) Q.

↓

using this Genotyping can be done

Gold std for MTB for genotyping is IS 6110 typing
This is not for M. Bovis

M. Bovis → Sporolotyping

(spacer oligonucleotide) → we see here repeats

TRANSFER OF GENE

seen in bacteria

✓ Transformation.

✓ Transduction

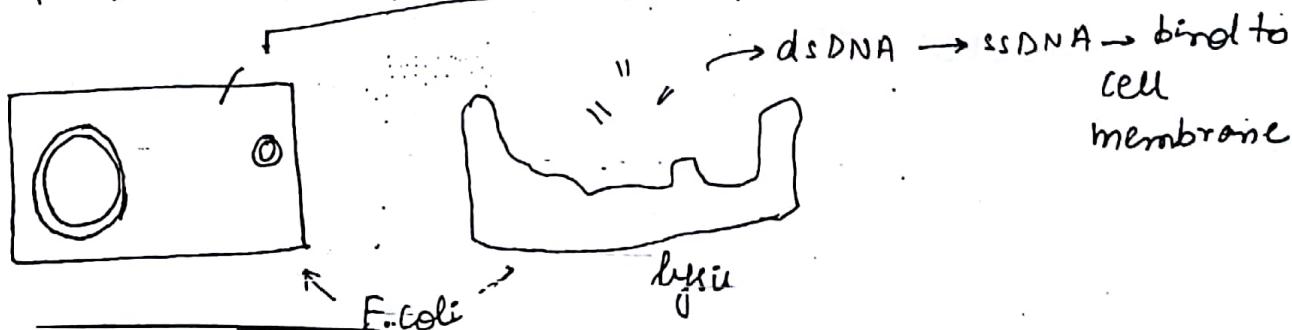
✓ Conjugation

TRANSFORMATION

Transfer of gene from 1 bacteria to another by naked DNA

↓

fragment of DNA after lysis of bacteria



Homologous Recombination

Replacement of a part of 1 strand in bacterial genome by ~~naked~~ DNA fragment at similar genes or alleles.

Griffith - Live type II non-capsulated Pneumococci (R)

↓
injected into mice → no pathogenicity.

Killed Type I capsulated Pneumococci (S)

↓
injected into mice

↓
no pathogenicity

When both were mixed together & injected into mice

↓

Death

↓

Bacteria is cultured

↓

Live Type I capsulated pneumococci

↓

He coined the term Transformation.

This was called Pioneering experiment in genetics.

Transformation is used in Recombinant DNA Technology.

→ Antigens

→ Vaccines

Drug resistance

Transformation may lead to ~~to~~ drug resistance.

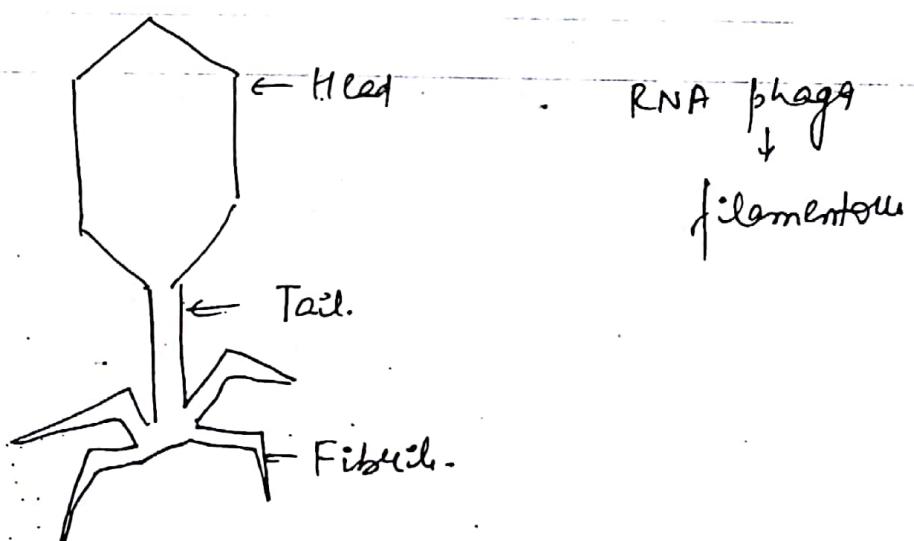
TRANSDUCTION

M/c method of gene transfer

Transfer of genes from 1 bacteria to another by
Bacteriophage



virus infecting bacteria



RNA phage
↓
filamentous

[DNA phage] can be used for bacteriophage typing

Q E phage do not carry out transduction?

- a) Lytic
- b) Lysogenic
- c) temperate
- d) lambda

Q. Σ phage shows lysogenic to lytic phase conversion?

as ① Temperate phage : lambda

Q. Σ phage do not show lysogenic to lytic phase conversion?

Lysogenic phage

Q. genes associated with galactose metabolism is transferred by Σ phage

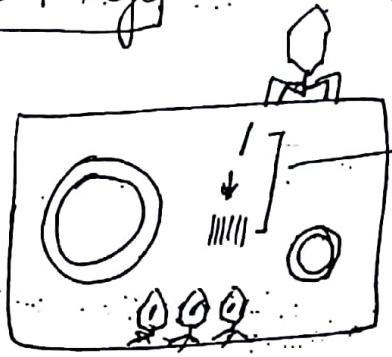
Lambda phage

Q. Sensconversion in salmonella is

- a) transformation
- b) transduction
- c) Lysogenic
- d) conjugation

Lytic Phage:

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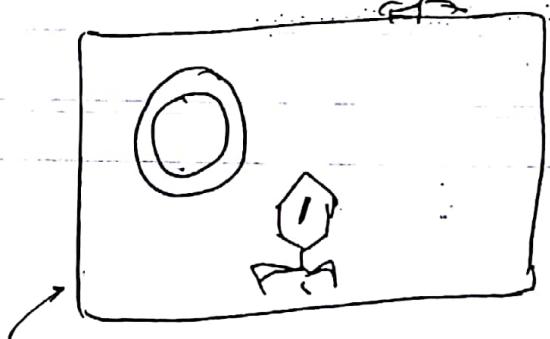
Transcription [from 1 genome to multiple genome]
- protein synthesis
packaging

No transduct occur normally → if error is not there

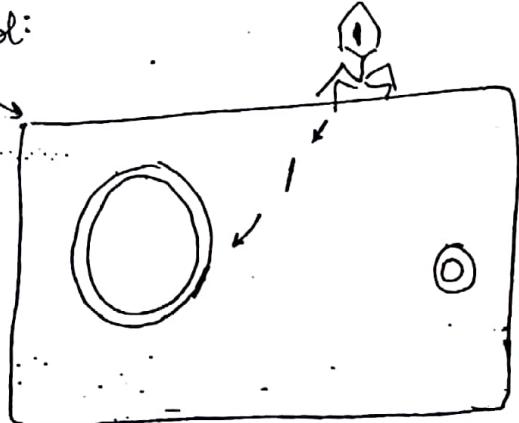
In case of errors while packaging, if bacterial genome enters ~~viral~~ viral —



It goes to other bacteria. → inserts the transfer the gene to this bacteria.



E.Coli:



Generalized Transduction :-

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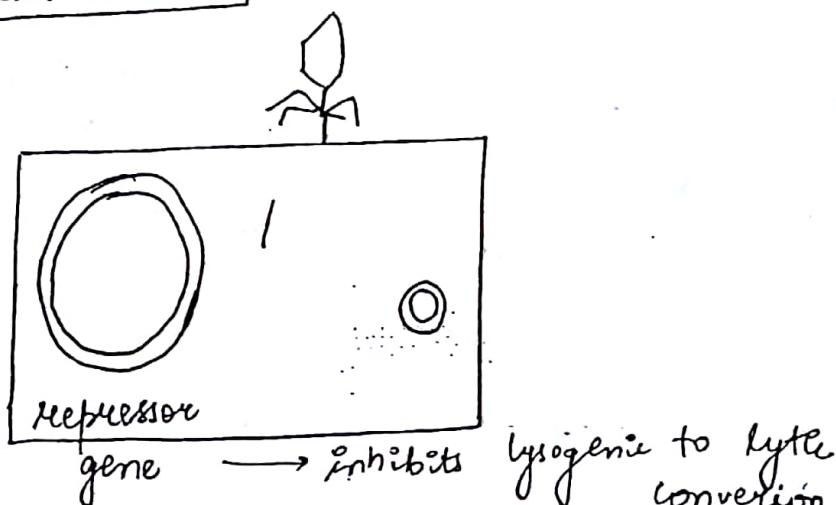
- lytic phage
- error in packaging
- Homologous recombination
- every ~~site~~ gene has equal chance of transfer.

Lysogenic Phage :- No Transduction

- Incorporation of phage DNA \rightarrow into bacterial genome
 by homologous recombination
 \downarrow [Transfer from virus to bacteria]

Lysogeny

- Rare phenomenon
- Seen in certain bacteria
- Imparts new character to the bacteria
- No lysogenic to lytic phase conversion.
 \downarrow
- No transduction.



Repressor gene is stimulated by lysogenic

Seroconversion in Salmonella

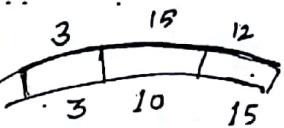
S. Anatum O_{3, 10, 12} H₆:e
 ↓ Phage 15

S. Newington O_{3, 15, 12} H₆:e

↓ phage 34

S. Minneapolis O_{3, 15, 34, 12} H₆:e

ANM



for Serotyping → use monovalent 'O' antisera.

~~for~~ Polyclonal is used for identification of bacteria's a whole (e.g. salmonella)

Corynebacterium Diphtheriae

β phage → tox gene

↓
'toxin'

due to lysogenic phage, β phage imports tox gene into corynebacterium leading to toxin producⁿ.

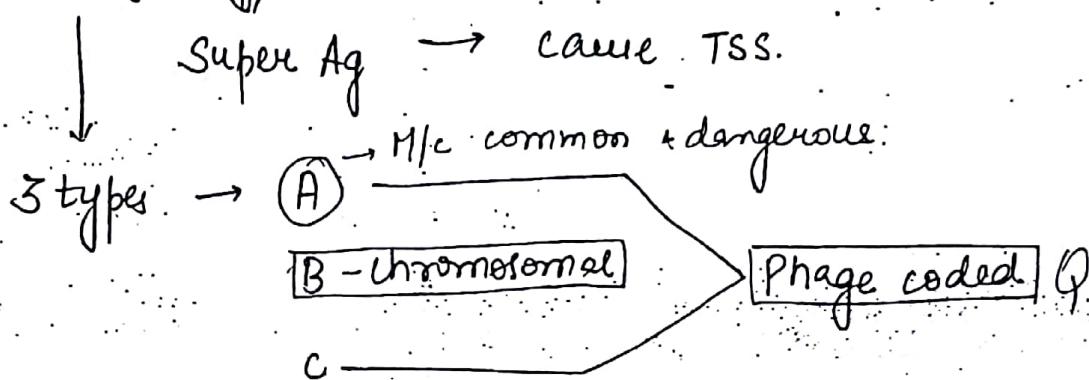
then it gets released toxin, hence "pathogenic".

Hence we do Toxigenicity test in Lab A of corynebacterium.

3) G+ A Streptococcus (S. pyogenes)

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Pyrogenic exotoxin (erythrogenic toxin)



Scarlet fever.

TSS in Group A streptococcus leads to scarlet fever.

If lymphangitis occurs → ~~Severe~~ Severe scarlet fever

Scarlet fever w/o lymphangitis → mild

4) V. Choleræ

5) Shigella

6) C. botulinum

} exotoxins.

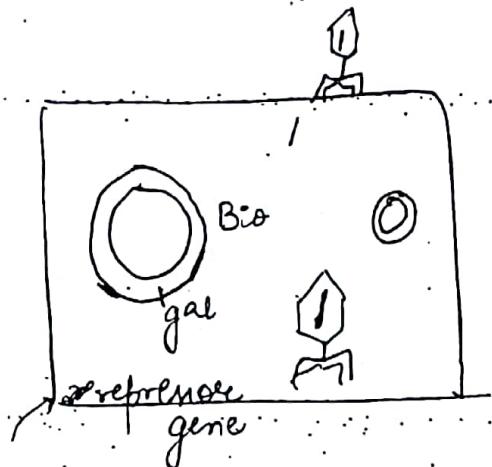
Temperate Phage ⇒ Transduction (+)

They show Lysogenic to Lytic conversion

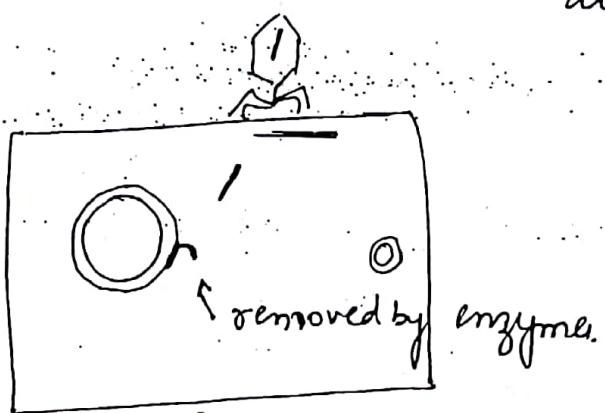
e.g. Lambda phage → insert phage DNA betw gal + bio genes



Galactose metabolism



E. coli



Specialized or restrictive transduction

- Temperate phage
- error in excision
- Homologous recombination seen
- Specific genes transferred

Lambda → genes associated with galactose metabolism

when bacteria starts dying
(repressor gene stops acting)

↓
Phage proteins are formed.
genetic material is taken out
↓
due to error bacterial genes
are also detached along with
viral genome (error in excision)

Phage infects other E. coli

But due to more similarity to bacterial genome
only bacterial genome is inserted into the

bacterium & viral genome is removed
by enzymes

Transduction

Transduction also can lead to Drug Resistance

CONJUGATION

Transfer of genes from 1 bacteria to another by Plasmid

Q. Transfer of chromosomal genes by conjugation is seen in -

a) F⁺

b) F⁻

c) Hfr

PG1 - Hfr, F'

d) F1

NBE - Hfr.

If 1 Hfr (cell 1) having A, B, C + D genes after the plasmid conjugates \bar{c} 100 F⁻ bacteria. (cell 2)
then predominant genotype would be

a) cell 1 \bar{c} A, B, C, D

b) cell 1 \bar{c} A

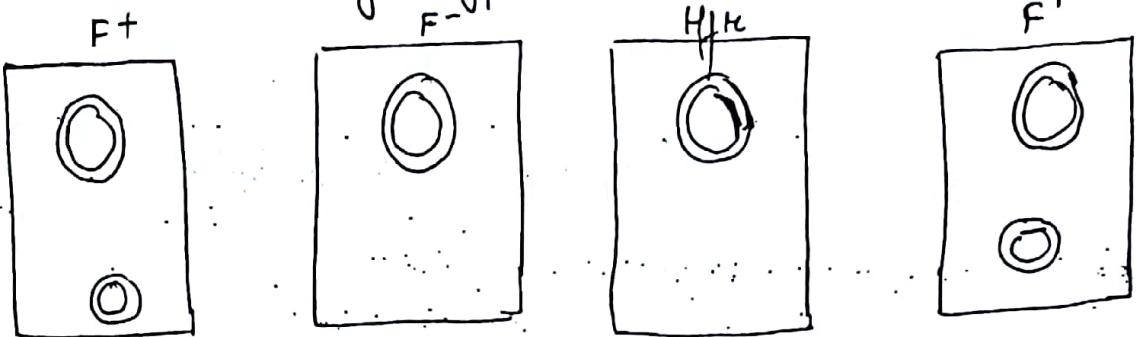
c) cell 2 \bar{c} A, B, C, D

d) cell 2 \bar{c} A

Since no change in genotype

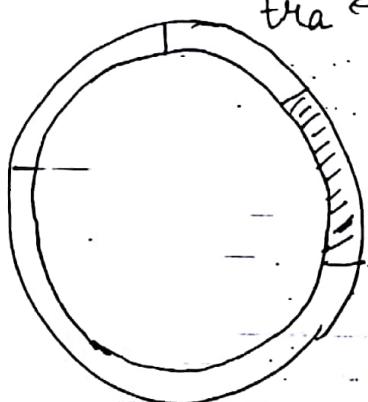
[F⁻ remains the same].

There are 4 genotypes in bacteria



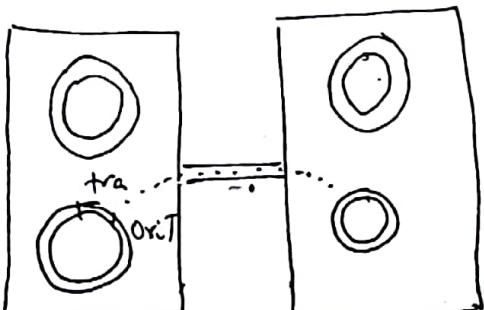
F plasmid

F' is the bacterium where plasmid contains chromosomal material



$tra \leftarrow$ transfer factor
 \downarrow
 $OriT \rightarrow$ origin of transfer
 \downarrow
 cuts outer strand after the gene.
 \downarrow
 LINEAR.

$F^+ \times F^-$



Plasmid is self replicating hence 1 strand forms the other

- ⇒ change in genotype.
- No. Homologous recombination is not seen. Q.

\Downarrow
 No transfer of chromosomal genes

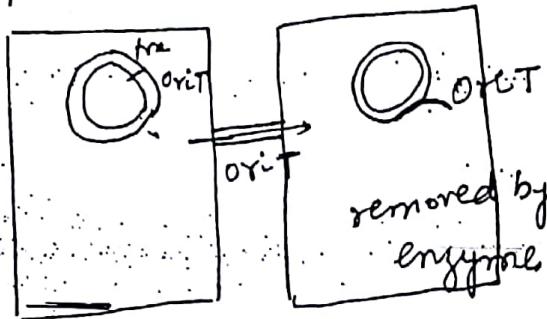
- Horizontal Transfer.
- Rapid

Plasmid mediated Drug Resistance.

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Hfr x F⁻

conjugation tube is stable $\approx 10\text{ min}$



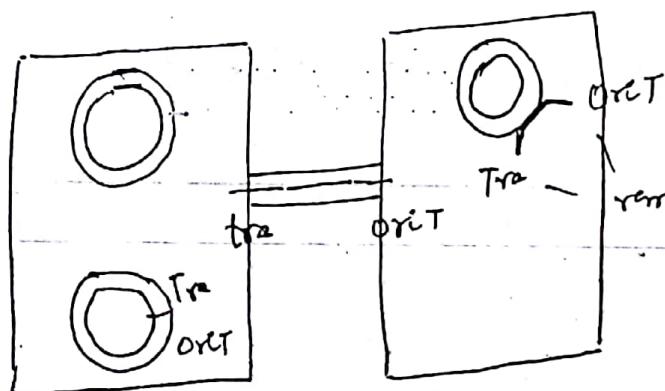
\rightarrow No change in genotype
Homologous recombination is seen

\rightarrow Transfer of chrm. genes

\rightarrow Horizontal \rightarrow not rapid

F⁺ x F⁻

- Sexduction.



\rightarrow No change in genotype
Homologous recombination is seen

- Transfer of chromosome genes

- ↓ frequent

F⁺ x F⁺
Hfr x F⁺

conjugation X

STERILIZATION & DISINFECTION

Killing of all organisms including spores.

↓
Biological Indicators (BI)

+
 10^6 species of *Bacillus* sp.
↓

] Quality control.

STEAM STERILISER (AUTOCLAVE)

121°C for 15 min at 15 lb pressure

- surgical equipment.

- dress material

- bandages

- culture media except L.J.

Loeffler's Serum Slope

↓ By Inspection

80°C for 1 hr x 3 days.

fractional
sterilization.

Tyndallisation

100°C for 1 hr x 3 days.

Sugar media → Heat at 100°C

BI → *B. stearothermophilus*

Once a week.

Bowie Dick Test or Vacuum leak Test
chemical indicator → every min.

measures the penetration of steam.

Briouen's tube - chemical

31

HOT AIR OVEN

160°C for 2 hrs.

→ glassware

→ Liquid

→ Sharp equipments. → chemical disinfectant can also be used for sharps

↳ autoclave (X)

BI → B. Subtilis subspecies NIGER

↓

B. atrophaeus (new name)

ETHYLENE OXIDE

Warm cycle → 50°C ± 5°C

Cold cycle → 37°C ± 5°C

f plaster ware (syringe, IV tube, catheter, urine bag)
t gloves

Gamma rays are preferred.

ETO → Heart Lung Machine

BI - Bacillus Subtilis GLOBI GI

IONISING RADIATIONS (cold sterilization) 32

γ rays - plastic
gloves
catgut sutures

BI \rightarrow **B. subtilis**

UV rays \rightarrow Biosafety cabinets

↓
HEPA filter Highly efficient particulate
size $(0.3 \mu\text{m})$

ULPA filter (ultra low particulate size
 $(0.12 \mu\text{m})$)

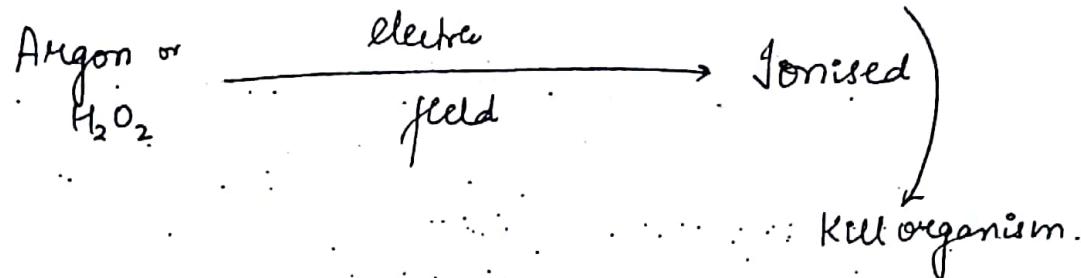
FILTRATION

- Vaccines + anti-sera
- Membrane filters $\rightarrow 0.22 \mu\text{m}$.

BI \rightarrow **Brevundimonas diminuta** (pseudomonas)

Bubble point testing.

GAS - PLASMA STERILIZATION



BI - B. atrophaeus

DISINFECTION

Reduction of no. of pathogens including Spores

ANTISEPTIC

Chlorhexidine

SPAULDING CLASSIFICATION

It classifies equipments

Critical → tissue contact → surgical equipments → Sterilisation.

Semimcritical - mucous membrane → endoscopes / colonoscopes (heat sensitive)

↓
2% Glutaraldehyde for 20 min
(CIDEX)

Laparoscope → 2% Glutaraldehyde for 2 hrs
(critical)

Non-Critical \rightarrow Skin \rightarrow Thermometer \downarrow
 intermediate level disinfectant
 Ethanol.

CLASSIFICATION OF DISINFECTANTS

Level	Sporecidal	Virucidal Non-enve	enveloped	Others
High	+	+	+	+
Intermediate	-	+	-	+
Low	-	-	+	+

High \rightarrow 2% Glutaraldehyde
formaldehyde

\downarrow
 fumigation \leftarrow

Paracetamol + H₂O₂

Intermediate \rightarrow alcohol
Phenol
Cl₂ releasing agent

Low → Quaternary ammonia
Lysol (cresol + soap)

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EFFICACY OF DISINFECTANT

- 1) Concentration
- 2) pH
- 3) Contact time
- 4) Organic compound → ↓ efficacy except phenol

Sputum } 5% phenol. 18 hrs
Stool } ↓
[RNTCP]

TESTING OF EFFICACY

→ Phenol co-efficient

Reidel Walker

not used

Chick Martin (organic matter)

nowadays

→ In use test (MIC)

→ Kelsey Sykes capacity test

In case of organic matter, this test can be done.

Endoscopes → 1st rinsed in H_2O to remove organic matter

↓
disinfectant

Complication

↳ M. chelonei abscess

PRIONS → autoclave at 134°c for 5 hrs.

or

✓ 2N NaOH.

IMMUNE SYSTEM

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Attribute	Innate	Adaptive
① Response Time	min/hour	Days
② Specificity	Low (for str. shared by group of microbes)	High (for specific Ag of microbe; every epitope)
③ Diversity	Limited	High: 10^8 to 10^{10} idotype
④ Memory	Low (only in NK cells)	High
⑤ Self/non self discrimination	Yes	Yes
⑥ Anatomic physical barriers	skin, mucosa, chemical (lysosome, IFN α & β , temp. & pH)	L.N., spleen, MALT
⑦ Blood protein	Complement	Antibodies
⑧ Cells	Phagocytes, monocytes, macrophages, neutrophils, NK cells, other leukocytes, epithelial, endothelial cells	Lymphocytes other than NK cells

Innate & adaptive immune response are interdependent
& not independent

MATURATION OF CELLS

occur in 1° Lymphoid organs

↓
Bone Marrow

Thymus

Antigenic exposure

→ 2° Lymphoid organs

↓
Spleen → blood borne

L.N. → lymphatic draining tissue

MALT (mucosa associated lymphoid tissue)

CALT (cutaneous " ")

Intestinal pathogens

↓
Skin.

Bone marrow act as 2° lymphoid organs in few cases

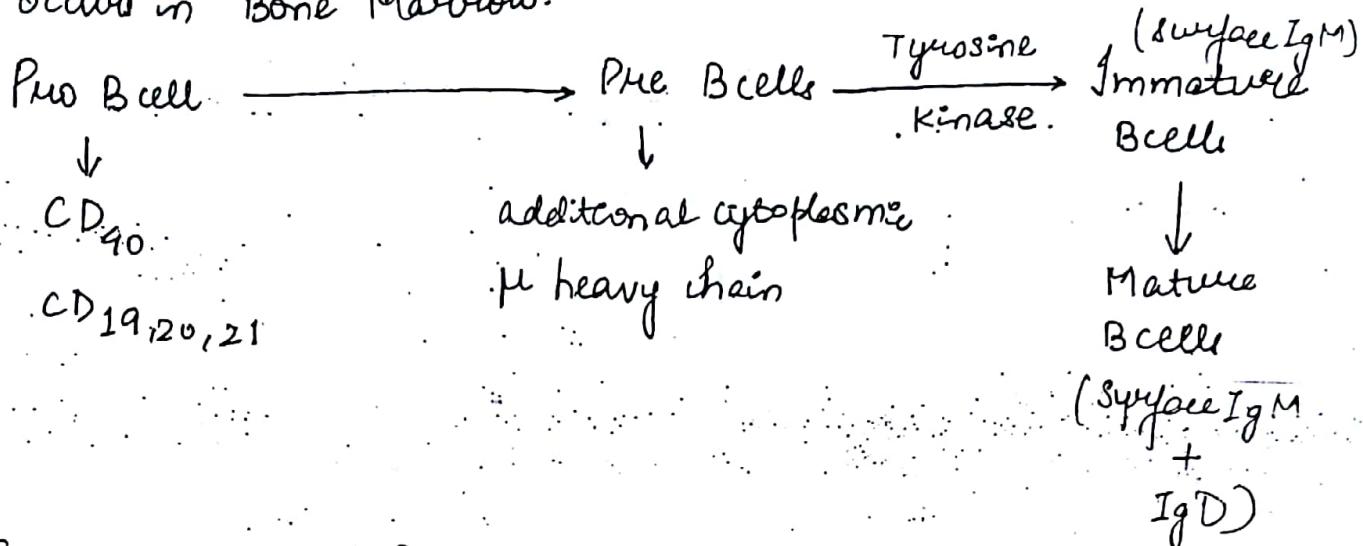
e.g. *Salmonella*

* Organ can act as both 1° & 2° lymphoid organ - Bone Marrow

B CELL MATURATION

39

occurs in Bone Marrow.



Follicular cell of Bone marrow.

↳ carries out the ~~feedback~~ -ve feedback selection.

- NO MHC

- They check the maturation \Rightarrow +ve selection.

↓
sent to 2° lymphoid organs.

Few B cells

↓
auto Ab.

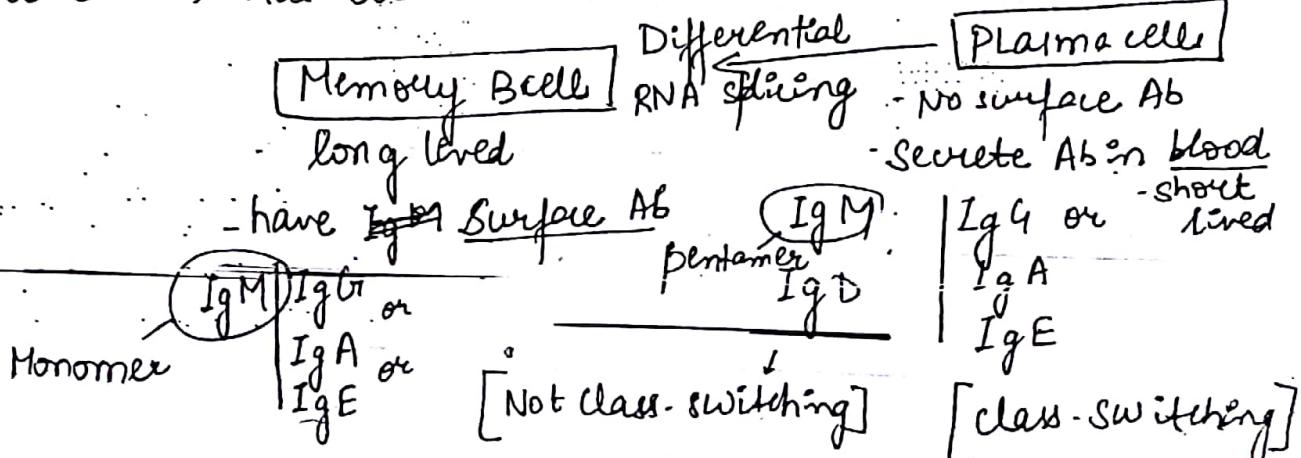
killed by apoptosis

↓
-ve selection

prevents autoimmunity

antigen exposure

Hence B cell, has both +ve & -ve selection.



1 plasma cell \rightarrow 2000 Ig/sec 40
↳ short lived

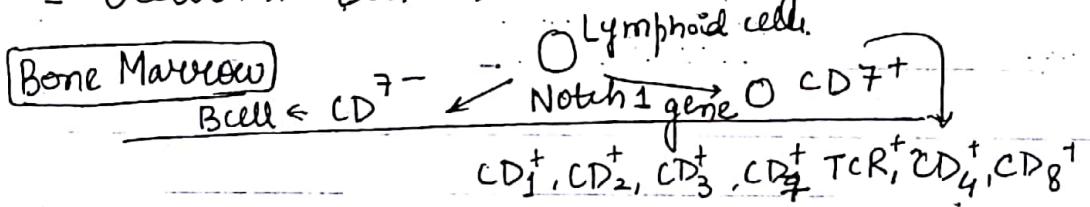
Q. One B cell \rightarrow 1.5 to 3 lakhs surface Ig
(mature) \rightarrow IgM & IgD
 $1:1000$

→ Surface Ab are monomeric

Peak Response Time in 1° Immune Response = 7-10 days
Secondary " 3-4 days

T CELL MATURATION

- Occur in Bone Marrow & Thymus



cells expressing
 CD^{7+} go to
thymus

Cortex

Positive Selection \rightarrow ① maturation
TCR $\xrightarrow{\text{Self MHC}}$ self MHC recognition.

$CD_3^+, CD_2^+, CD_3^+, CD_7^+, TR^+ \xleftarrow{CD_4^+ CD_8^- : TH}$
 $CD_4^- CD_8^+ Tc$

Medulla

95% \rightarrow Negative selection \rightarrow few T cells have TCR \approx ↑ affinity for self MHC.

Killed by apoptosis

2° Lymphoid organs

prevents autoimmunity

APC → MHC II & MHC I (nucleated cells)

41

Professional [Dendritic cells] → specialised



→ Macrophage

→ B cell

Non-Professional

→ Microglial cell (brain)

→ fibroblast on skin

→ pancreatic β cells

→ vascular endothelial cells

→ epithelial cells — N cells

Thymic epithelial etc.



All nucleated cells or platelets have MHC I

MHC-I → has 1 chain

MHC-II → has 2 chains

If affinity of T cell is quite more toward self MHC

It may act as autoantigen

occurs in medulla

Hence undergoes apoptosis

LYMPHOCYTE HOMING

42

Thymus Dependent area

[Spleen] Periarteriolar Lymphoid Sheath

Thymus Independent area

cortical medullary
follicular.

[Lymph node] Paracortical area

germinal centre

T cell zone

B cell zone

(neonatal)

In a person undergoing thymectomy, at / adderence LN Biopsy

↓
Paracortical area undergoes atrophy

↓
as T cells are not formed.

TOLL LIKE RECEPTORS

TLR1 → Mycobacteria +
Gram -ve bacteria

TLR2 → Gram +ve bacteria

Mitochondria

Mycobacteria

Yeast & other fungi

Schistosomes

TLR3 → Viruses

TLR4 → Gram -ve bacteria

RSV

Fungi

43

TLR5 → Bacteria

TLR6 → Mycobacteria

Gram +ve bacteria

Yeast & other fungi

TLR7 → Viruses

TLR8 → Viruses

TLR9 → Bacterial DNA

Herpes virus

Malaria parasite heme byproduct

TLR10 → Unknown

TLR11 → Unpathogenic bact

Toxoplasma

TLR12 → Unknown

TLR13 → Vesicular stomatitis virus

ANTIGENS

Epitopes are found in antigen

44

\downarrow They are separately immunogenic

Antigenic Determinants

1) Size < 10 k Dalton → Haptan.

10-100 k Dalton → Haptens or Immunogens

> 100 k Dalton \rightarrow Immunogen.

All antigens are not immunogens but all immunogens are antigens.

2) Chemical Nature

Protein > polysaccharide > Lipid > Nucleic Acid

3) Susceptibility to tissue enzymes.

47 Foreignness.

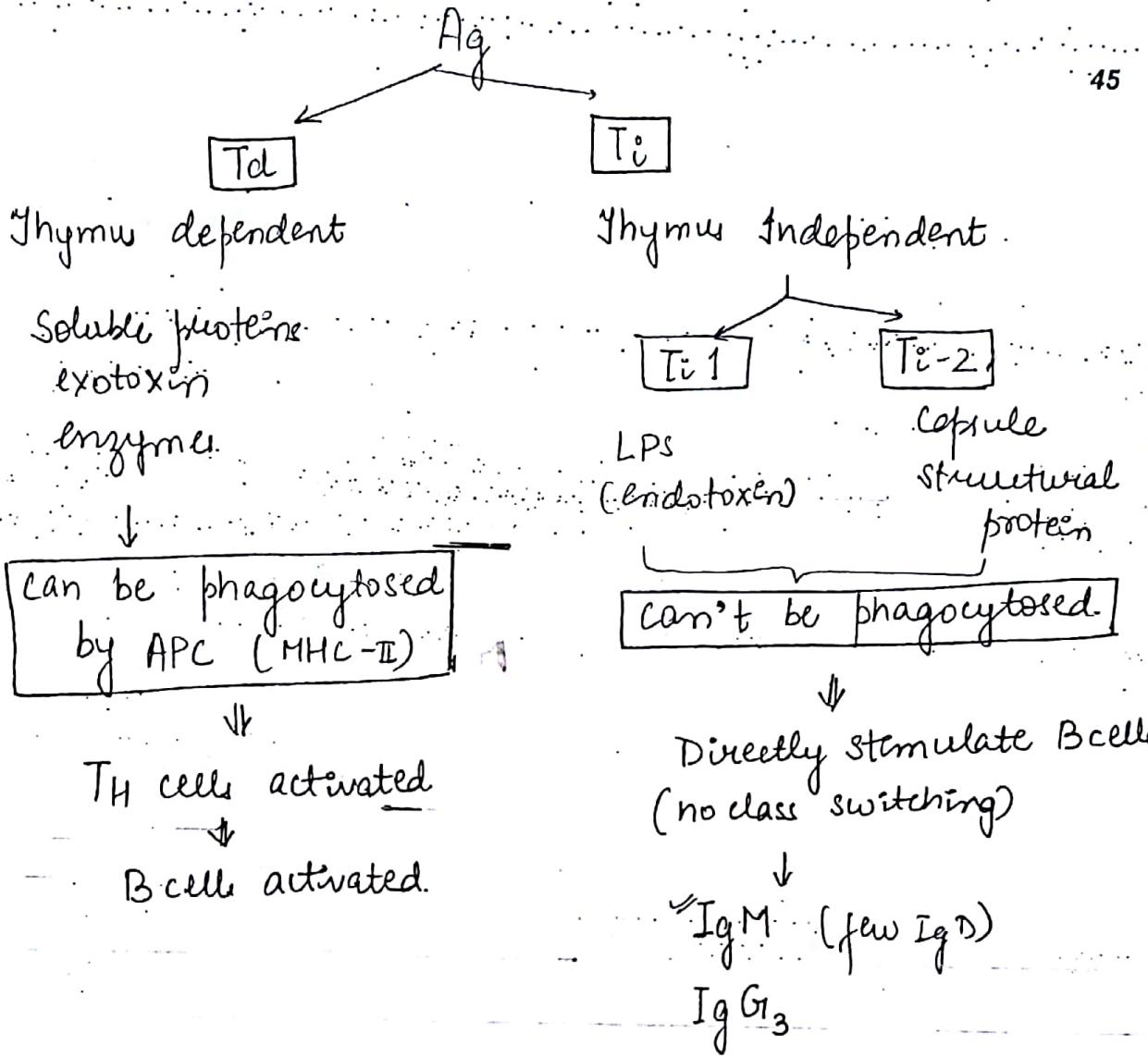
directly proportional

Sequestered Ag → lens protein
↓
specums

can lead to
autoimmunity

④ Immunoprivileged sites

liver
uterus } No immune response.
testes
Brain.

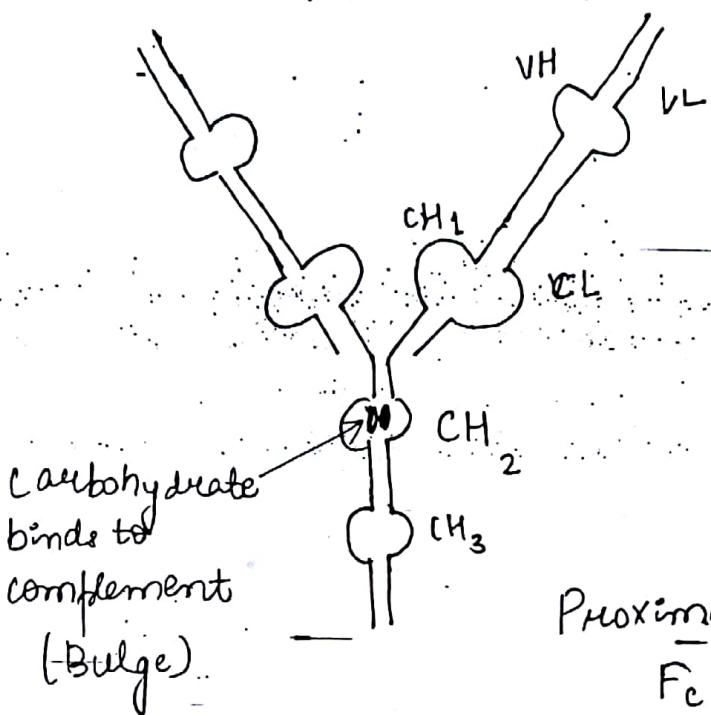


Td	Ti
Class switching	⊕
Ab diversity	⊕
Affinity maturation	⊕
Memory response	⊕
Phagocytosis	⊕
Complement activation	⊖
	⊕

B cell funⁿ on cytokines released by T cells.

46

ANTIBODY



distant (NH_2 terminal)

Fab → idotype

Proximal (COO⁻ terminal)
Fc → isotype

IgM, IgE → additional CH₄ domain.
Carbohydrate → CH₃

Molecular Wt

IgM → 900 kDa (millionare)

IgA → 365 "

IgE → 190

IgD → 180

IgG → 150

Max Carbohydrate content ⇒ IgE - 12%
IgD - 13%

* Idiotype is determined by variable domain of both H & L chain.

47

↓
Hyper variable region

[Complementarity Determining Region]

↓

each Domain has 3 CDR



3 Domain
CDR

Paratope is the portion of Ab where Ag comes to bind.

2 similar Ags can bind to both side.

Func" of Idiotype - Antigen Binding.

* Igotype is determined by constant region of mainly H chain & L chain

Func" \Rightarrow IgM \rightarrow complement activation

IgG \rightarrow opsonisation & dependent ADCC (antibody mediated cell mediated cytotoxicity)

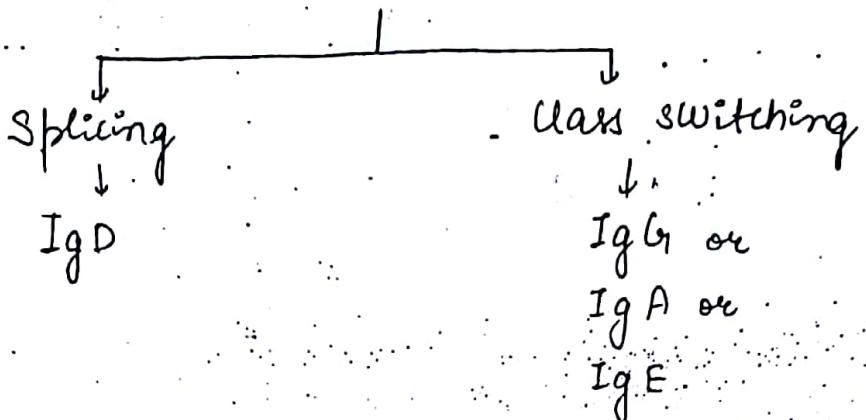
IgE \rightarrow degranulation of mast cells
basophilic, eosinophilic

IgG \rightarrow destroy cell membrane of helminthes

IgA \rightarrow mucosal immunity

IgD → no biological funcⁿ

MECHANISM OF ISOTYPE FORMATION:



Genes encoding

H chain

- chr 14

L chain (kappa)

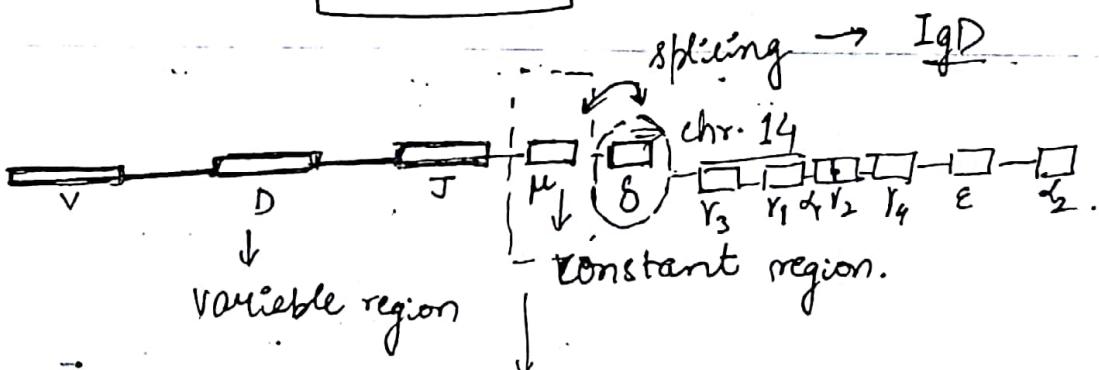
- chr 2

(Lambda)

- chr 22

Located on:

$$K:\lambda = 2:1$$



- Hence IgM is the 1st Ab formed

- Marker of acute infec.

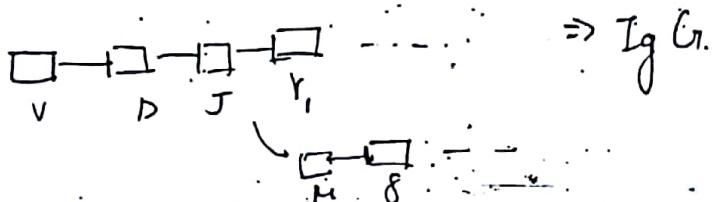
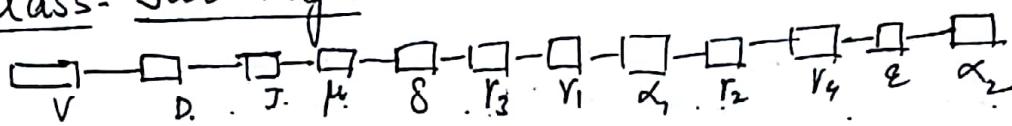
- Whenever B cell stimulated.

IgM is always formed

Class-switching occurs in B cell only once

49

Class-Switching:



- 1) enzymatic removal of isotype determining gene
↓
gene rearrangement

2) irreversible

3) occurs only once in one B cell

4) (TH_2) $(CD_{40L}) \longleftrightarrow (CD_{40})$ B cell

↓
B cell get activated.

5) Germinal centre of 2° lymphoid organs.

Nude Mice [natural strain]

- No hair
- Chr 11 defect on Fox N₁ gene

- vestigial thymus
↓
selective T cell deficient

- Gnotobiotic environment [germ free environment]
- IgM (few IgD) → No class switching

Di George Syndrome

Fish Mouth

Deformed ears

Wide set eyes

Cyanosis ← cong. heart Disease. [Fallot's M/c]

Seizure due to hypocalcaemia [no parathyroid]

No thymus.

Defect - [Chr 22 q 11] defect

- failure of development of 3rd, 4th pharyngeal pouch

No class switching → only IgM Ab formed

* Hyper IgM Syndrome
defect in CD_{40L} on T cell

51

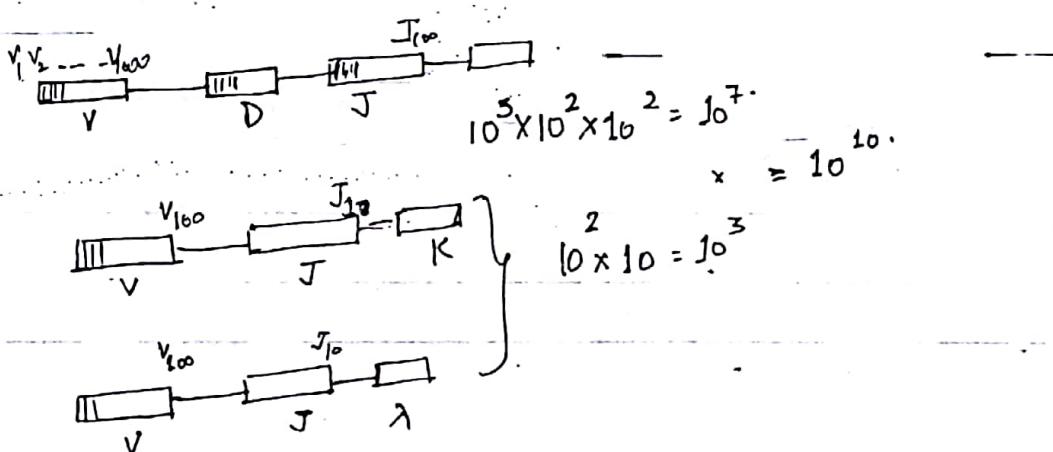
\downarrow
Selective B cell Deficiency

Classified on the basis of clinical outcome

- No class switching
- $\uparrow\uparrow$ Ig M $\rightarrow 10 \mu\text{g}/\text{mL}$ [N] $1.5 \mu\text{g}/\text{mL}$

Ab DIVERSITY

Mechanism of idiosyncrasy formation



Mer. Helminthic infecⁿ in HIV = Strongyloides.

- Recombination of multiple genes encoding for variable domain of both H + L chains

\Downarrow

Recombination of every H chain
in all different L chains

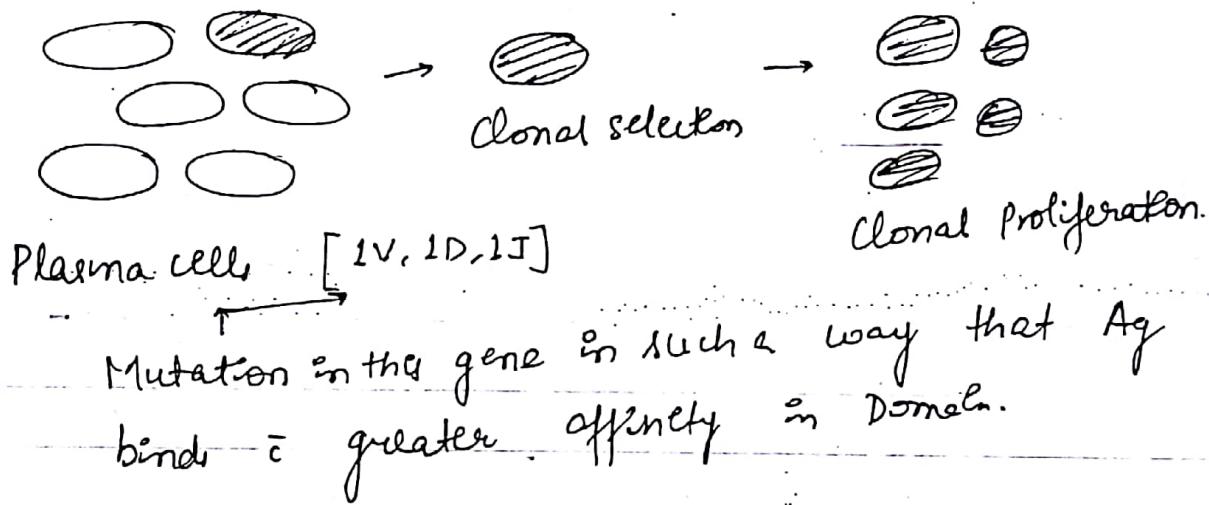
Nucleotides are getting added continuously, hence genes never get exhausted

Mutation also leads to addition of genes

1> Terminal nucleotide addition by tdt enzyme

2> Removal of nucleotide by exonuclease

AFFINITY MATURATION (Somatic Hypermutation)



By mutation in the variable gene,

↑ affinity

↓
Clonal selection of that cell
rest cell die.

↓
The cell undergoes clonal
proliferation

Through affinity maturation we can know about
present or recurrent infection

past infec" → high affinity
present " → low affinity

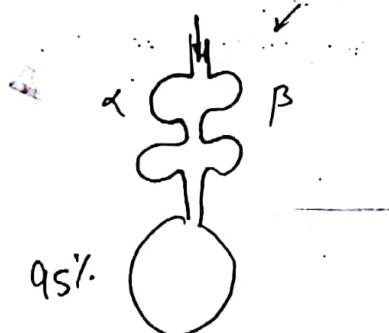
C Ab has ↑ affinity?

IgG → since time taken is more

IgM → ↓ affinity → but High affinity (valency) 10

Valency of B cell Receptor = 2

T cell Receptor = 1.



18/2/18

M/C Mechanism responsible for idiotypes or Ab diversity

= Recombination at the variable regions.

Affinity Maturation is due to mutation at the variable region genes.

C IgG isotype has highest affinity IgG

C " lower " IgM

- A pt suffering from CVID not susceptible to c Shigella species → Shigella dysenteriae.
- The B cell stage predominant in Bruton hypogammaglobinemia → Pre B cell
- The cytokine responsible for pathogenesis of salmonella gastroenteritis → IL8
- Negative selective helps in prevention of c type of disorders - Autoimmune

ALLOTYPES

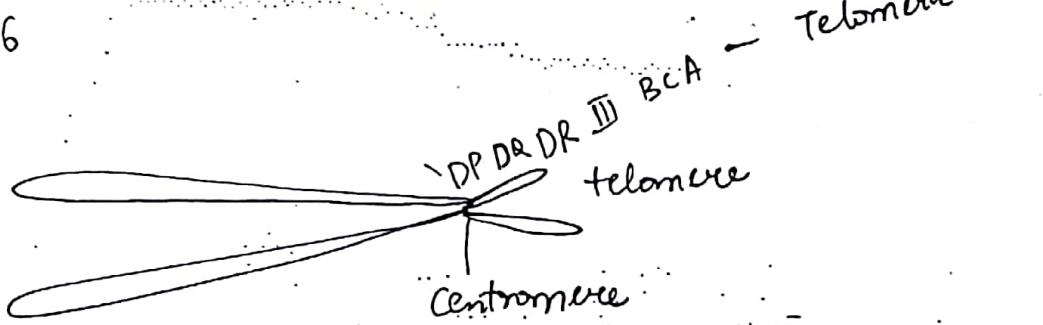
Subtle amino acid difference in the constant region of H chain & L chain.



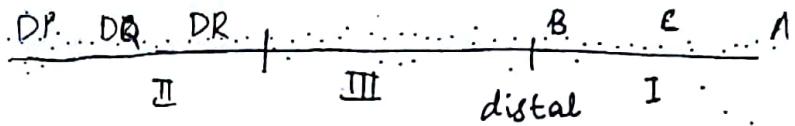
Allelic Exclusion

MHC [MAJOR HISTOCOMPATIBILITY COMPLEX]

It is a ~~set~~ cluster of genes located on short arm of Chr. 6

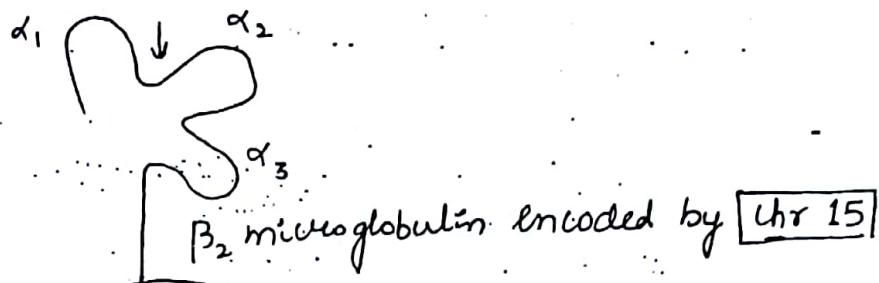


(CD Player
Centromere)



55

MHC-I



Site of binding of Ag in MHC-I \Rightarrow Distal α_1 , α_2 .

- Peptide of length 8-10 AA can bind to this site
- Deficiency of B_2 microglobulin leads to
 - Hereditary Haemochromatosis
 - Due to "mut" in 283 position of Hfe gene



Due to this mutation

Cysteine is replaced by Tyrosine

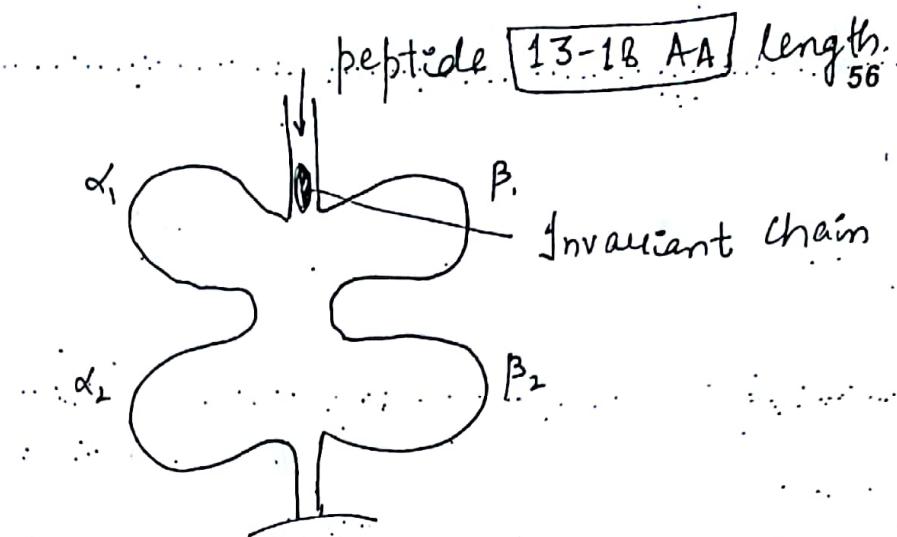


$[B_2$ microglobulin regulates "absorp" of Fe]



So Iron overload may occur

MHC-II



- Invariant chain prevents binding of normal peptide

↓
Hence MHC-II prevents autoimmunity

- Defect in Invariant chain leads to autoimmunity
[c is due to defect in DQ, DR region]

MHC-III

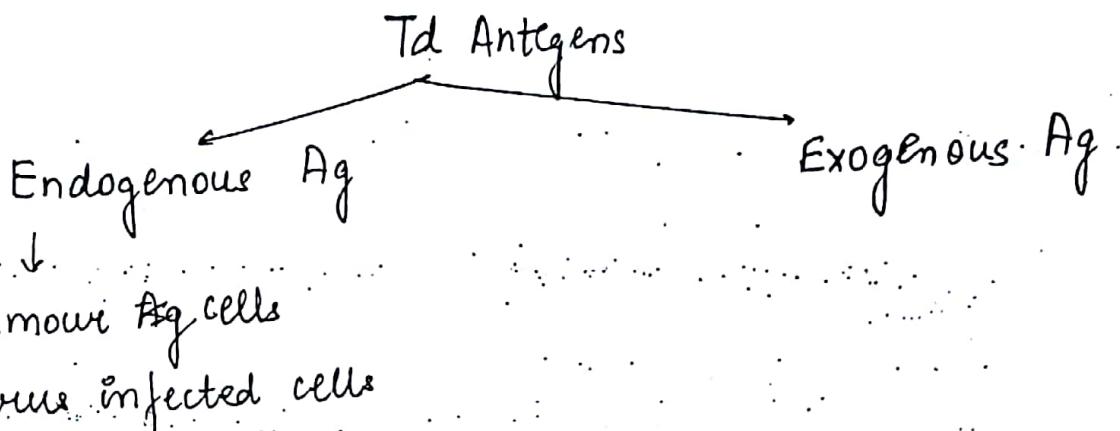
It codes for complement protein $C_4 + C_2$
Heat shock proteins

TNF $\alpha + \beta$

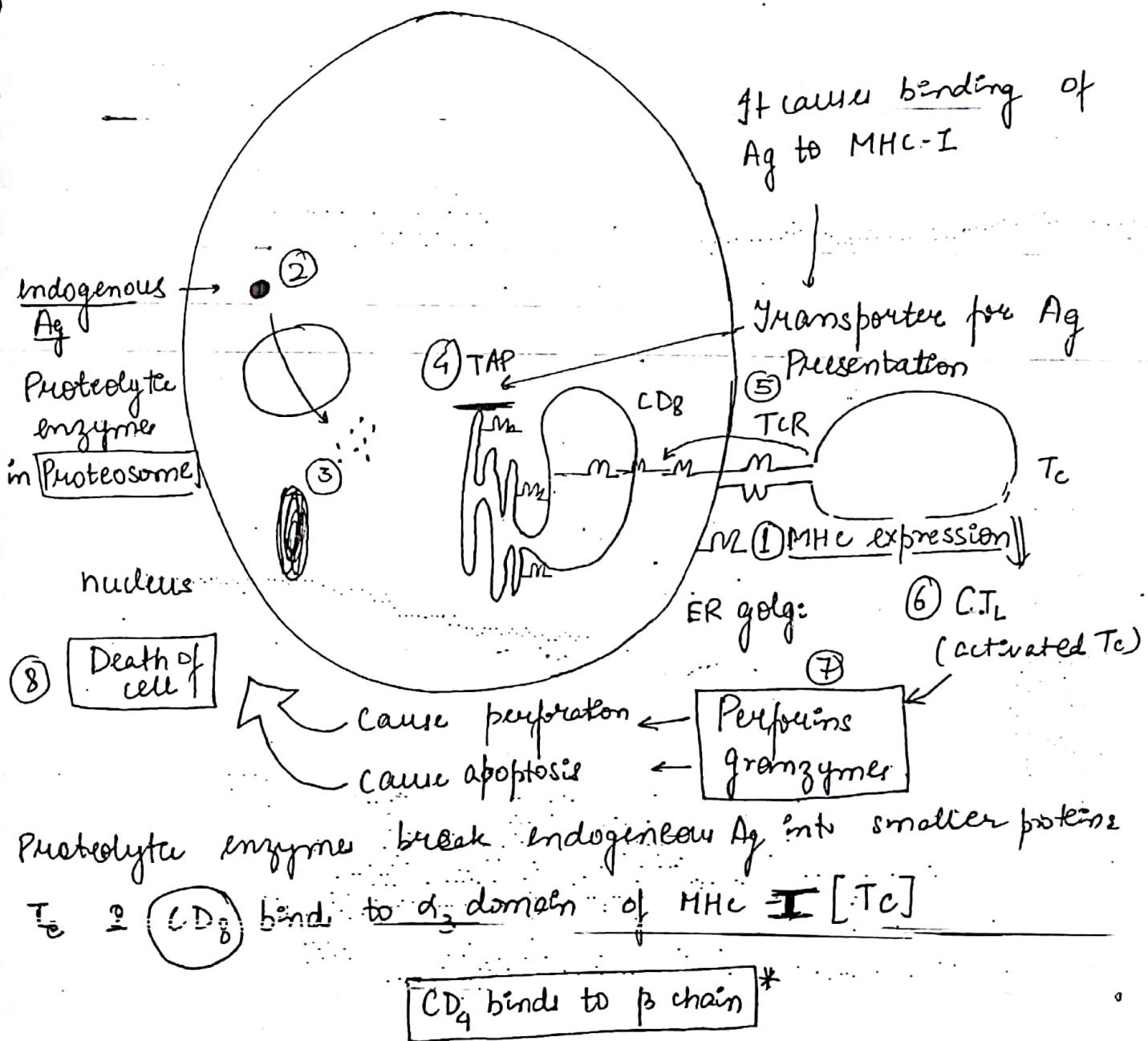
21 Hydroxylase

ANTIGEN PROCESSING (Td)

57

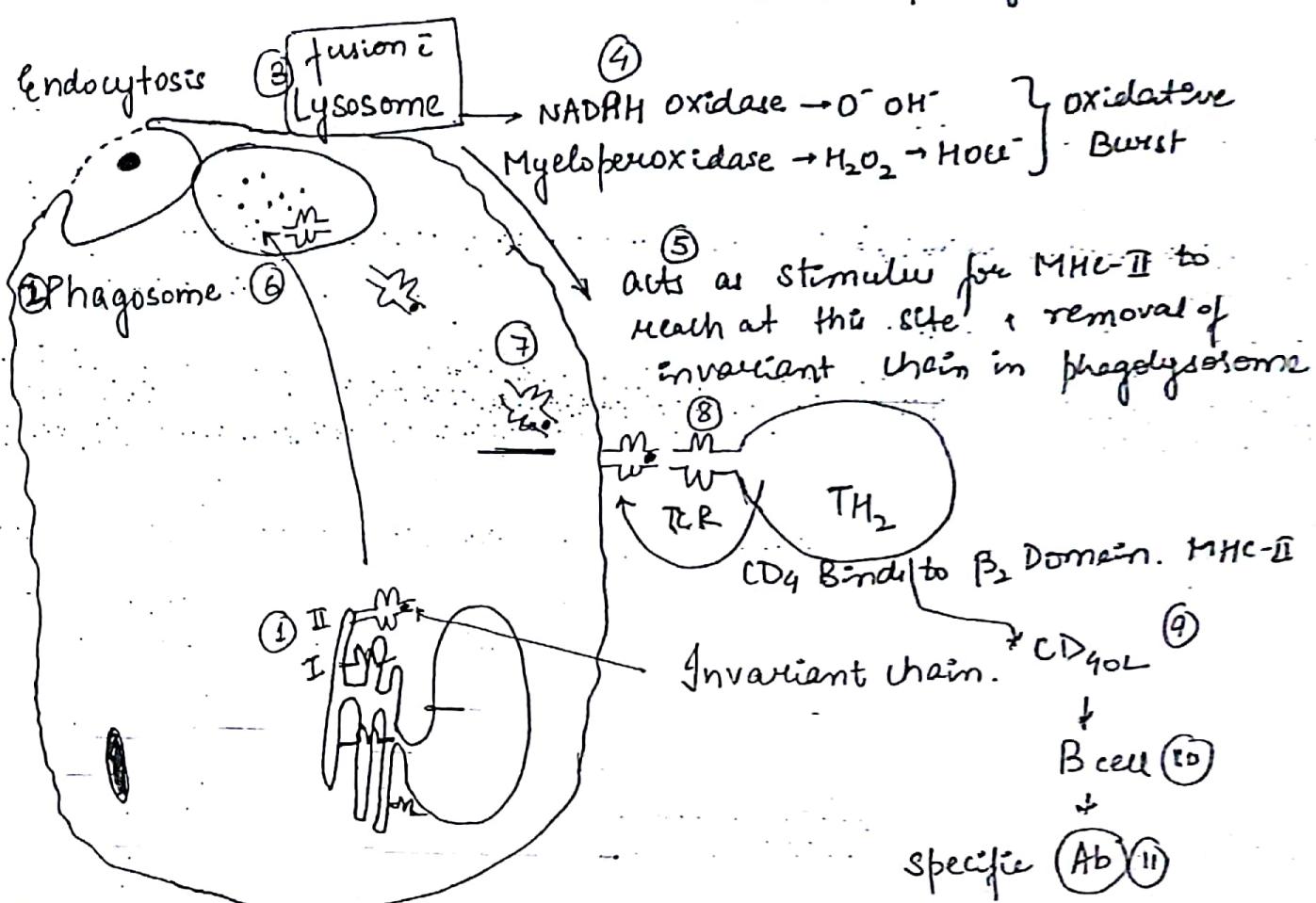


I) Endogenous Ag Processing



* Exogenous Ag Processing

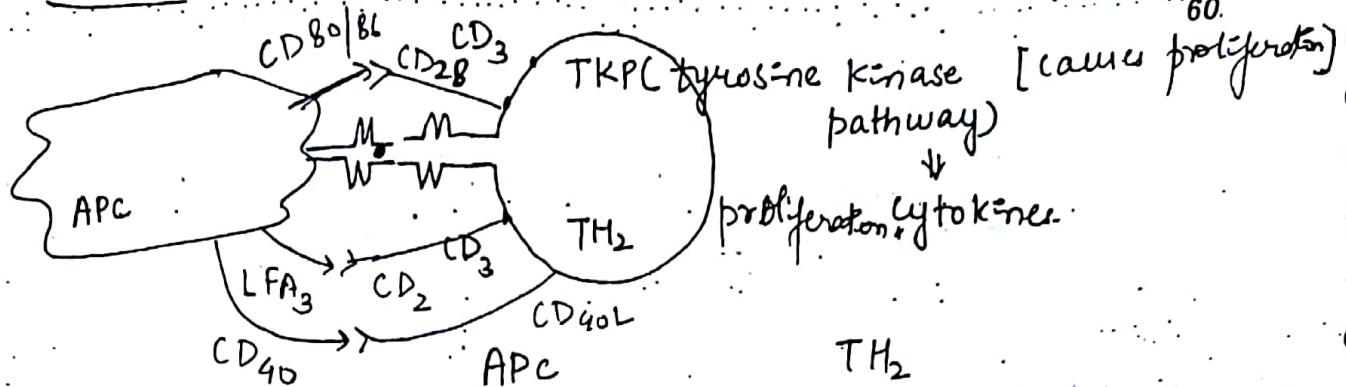
B cell → pinocytosis 59



SIGNAL TRANSDUCTION OF TCELL

SIGNAL

TRANSDUCTION



1st Signal

Ag Mh restricted T \leftrightarrow **TCR**

B₇ (CD80/86) \leftrightarrow **CD28**

**(costimulatory
Signal)**

LFA-3

\leftrightarrow **CD2**

CD40

\leftrightarrow **CD40L**

3rd Signal

Signal Transduction



CD3 activated



TKP activated



**Proliferation
cytokines ↑↑**

$\boxed{\text{TH}_0}$ [Naive TH cells]

IFN γ

IL4

$\boxed{\text{TH}_1}$

Produce

IL2

IL12

TNF β

IFN γ

activate macrophages
intracellular pathogens

IFN γ ↑↑

Neurosis

$\boxed{\text{TH}_2}$

Class switch
B cells

IL13

TGF β

IL4

IL5

IL6

IgE

IgA

Proliferation of
many cells

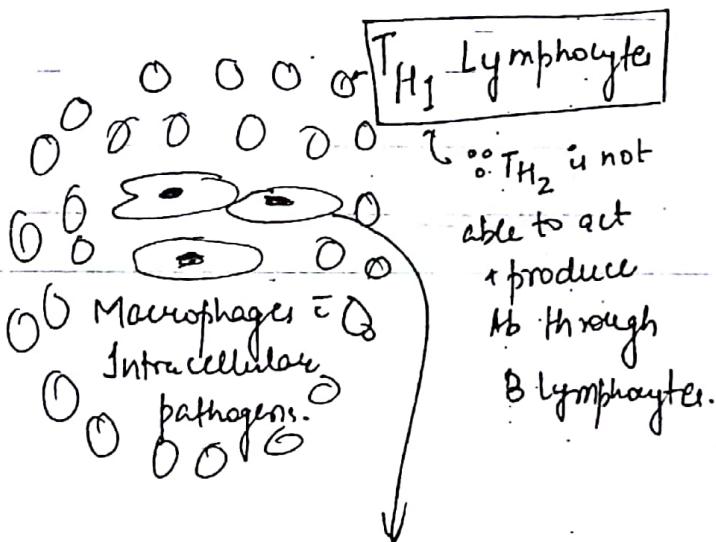
$\text{TH}_1 \rightarrow \text{IFN-}\gamma \xrightarrow{\text{B cell}} \text{IgG}$

$\text{TH}_2 \uparrow$

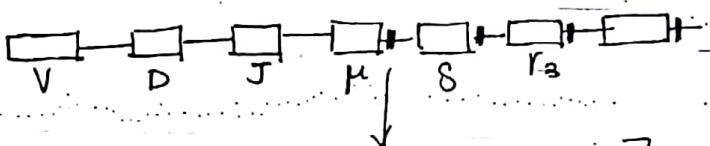
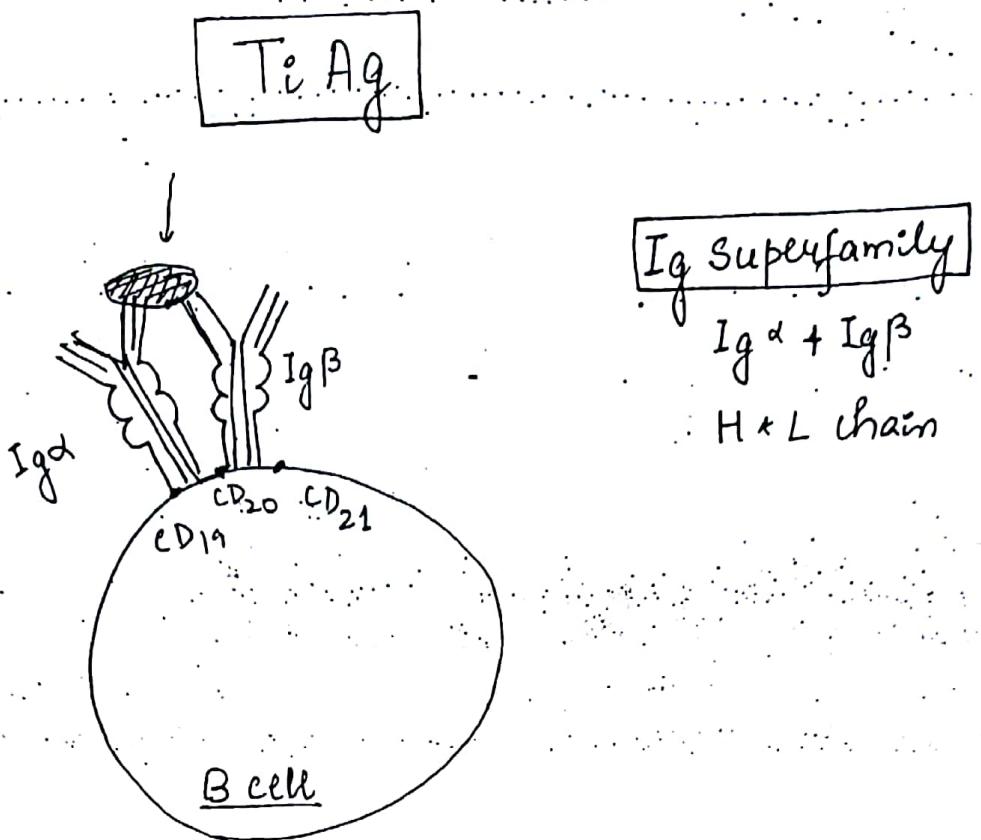
For formation of IgG,
 $\text{TH}_1 + \text{TH}_2$ both have to
play the role as
IFN- γ is released by
 TH_1

Plasma cells

↓
Humoral Immune
Response.

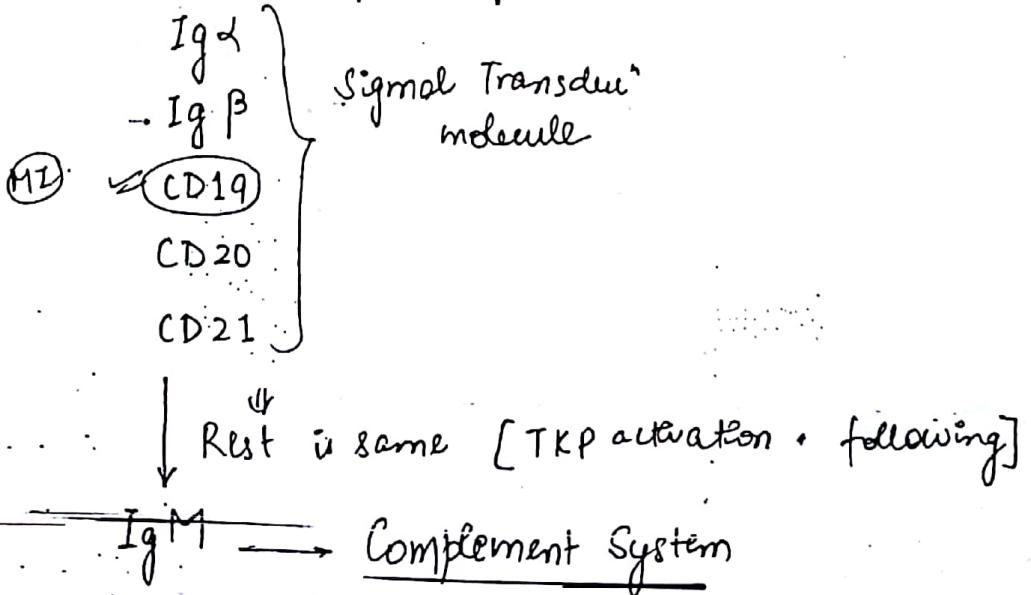


Cell mediated Immune Response



this gene encodes for
Ig α · Ig β chains] → Differential RNA (Plasma-Memory)
Splicing

Signal Transduction by T_i Ag



COMPLEMENT SYSTEM

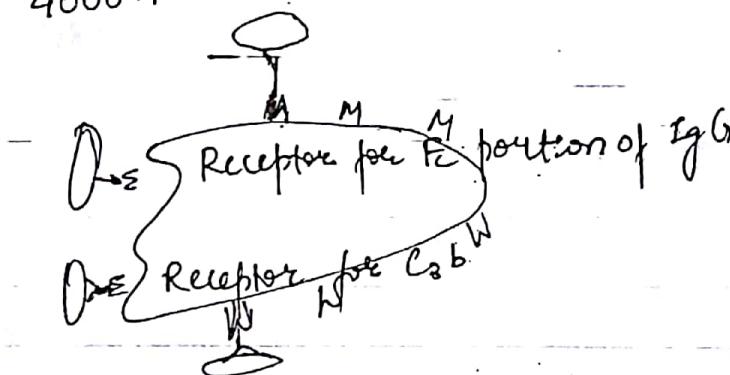
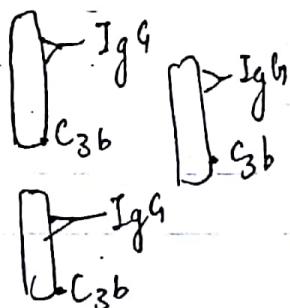
63

Functions :-

- 1) Opsonisation (C_{3b})
- 2) MAC mediated lysis of bacteria (C_{5b-9})
- 3) Immune complex clearance (C_{3b})
- 4) Chemotaxis by smaller fragments (C_{5a})
- 5) Ag - Ab complex activation
- 6) Memory

OPSONISATION

↑ phagocytosis $\Rightarrow 4000 \times$



Opsonins

Innate response

Collectins SPA,

SP-D,

MBL

L-ficolin

C1q

* C3b

C4b

C3b

cleavage products (iC3b, C3c, C3dg)

Adaptive response

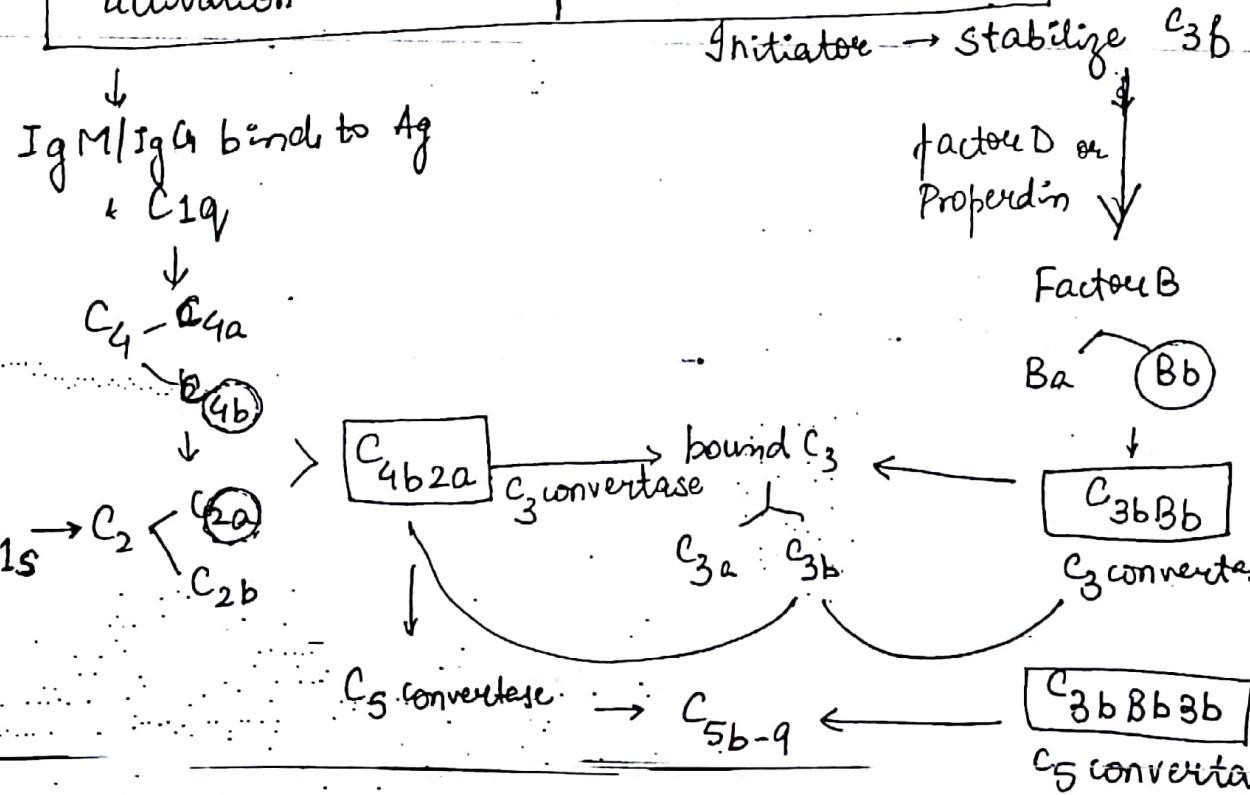
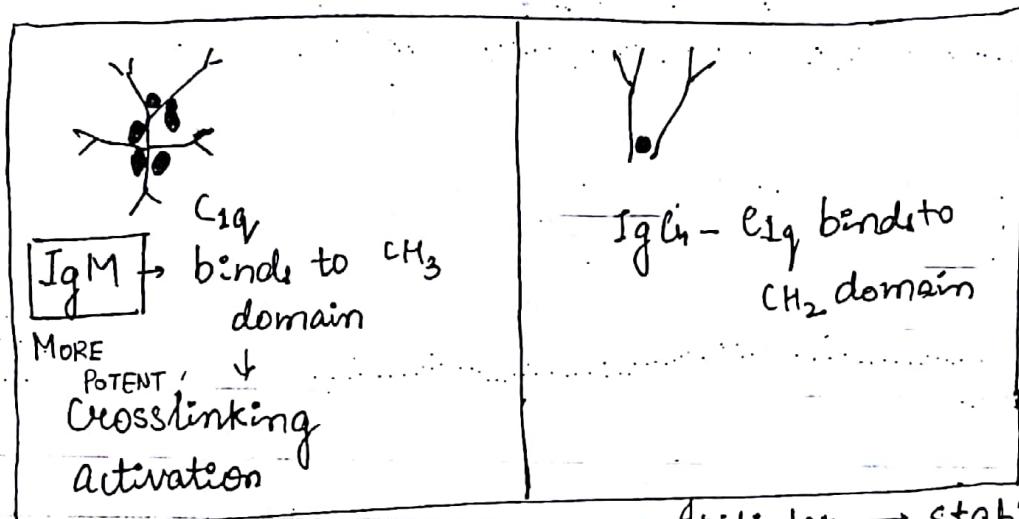
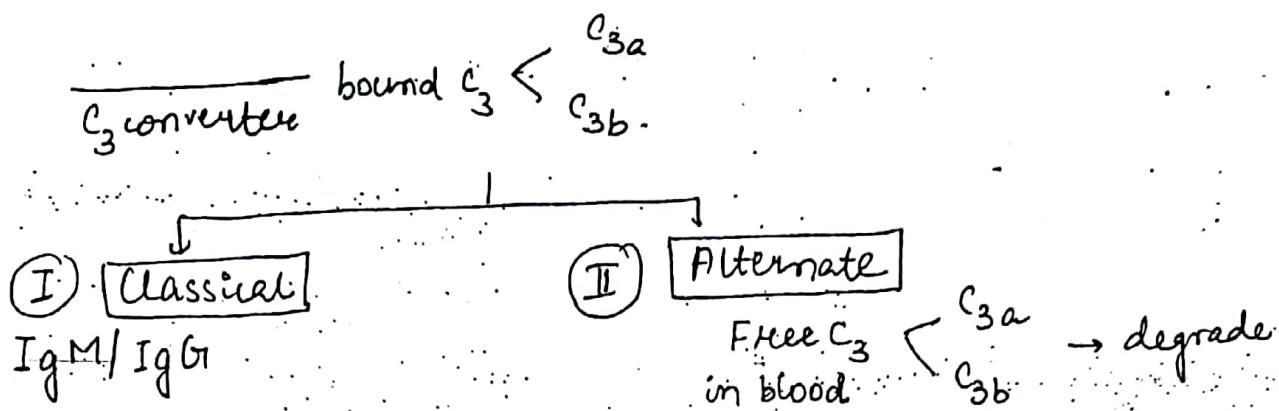
Ig A

(Ig G)*

CRP

Max. complement Protein \Rightarrow Bound C₃.

64



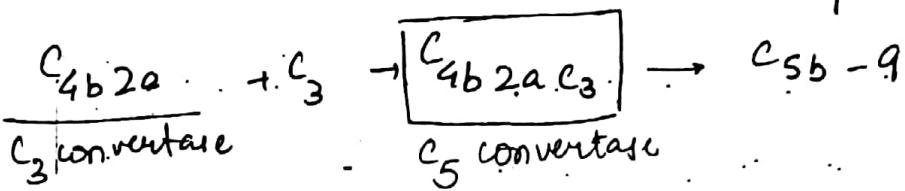
Neisseria Infⁿ ← Lysis

It doesn't allow opsonization.

MAC

65

So,



Initiators of Alternate

- 1) Endotoxin
- 2) Snake venom
- 3) Yeast peroxisome protein
- 4) Mannose on yeast
- 5) Nephritis factor
- 6) Dextran sulphate
- 7) Inulin
- 8) IgG, IgA, IgD.

C_{4b} binds to C_2 , expose it to the action of C_{1s}

C_{1s} cleaves C_3 into C_{2a}, C_{2b}

Yes Snake, In & End Ygy, kia
→ GLAD

IgE, IgG₄ doesn't activate complement

(1)

LECTIN PATHWAY

MBL (mannose binding lectin)

↓
It activates C1q directly

rest similar to classical pathway.

REGULATION OF COMPLEMENT

66

Protein

Function

as C1 Inhibitor → Dissociation & inhibition of $C_{1r_2}s_2$ from C_{1q}

2) Decay accelerating → Dissociation of C_3 convertase factor (CD55)

3) CR1

C4BP

Factor H

} → Dissociation of C_3 convertase
cofactor for factor I

→ cleaves $C_4b \circ C_{3b}$

as Factor I

5) Membrane cofactor of → cofactor for factor I
proteolysis

MCP

6) S protein or vitronectin → Binds soluble C_{5b67} & prevent insertion into host

7) Protectin (CD59) → Blocks binding of C_9 & formation of MAe
(carboxypeptidase N, B & R) → Inactivates the anaphylactic C_{3a}, C_{5a}

Deficiency of C₃ \Rightarrow No Opsonisation
(bound) \downarrow
Pyrogenic Infect.
GN

67

Deficiency of Late complement \Rightarrow Recurrent Neisserial Q.
Proteins Infection.
[MAC mediated Lysis ⊗]

Deficiency of Decay accelerating factor (CD55) \Rightarrow PNH.

Deficiency of C₁ inhibitor = Hereditary Angioneurotic edema



Swollen lips —
Painful abdominal edema
Laryngeal edema

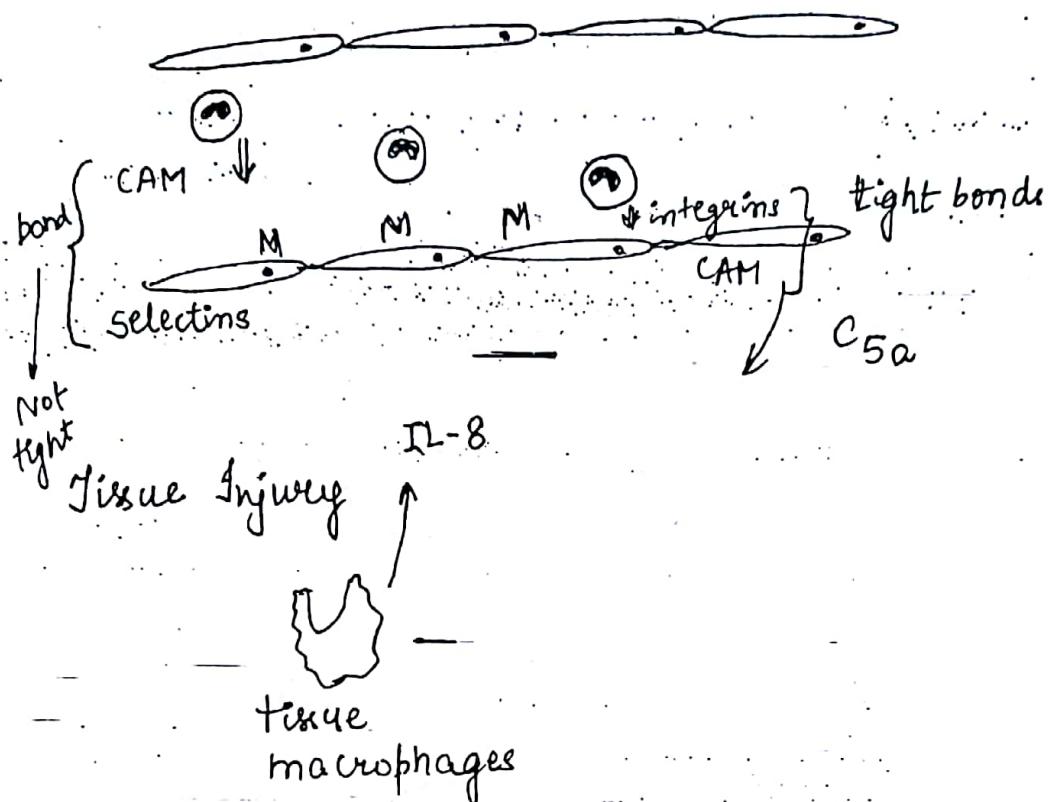
Δ - Best marker

↓
 $\downarrow C_4$ Level

PHAGOCYTOSIS

68

Extravasation of Neutrophils



Margination \Rightarrow Due to selectins \rightarrow **Rolling** \rightarrow **Adhesion**

fucosyl transferase enzymes \downarrow β_2 integrins

Chemotaxis \leftarrow **Diapedesis**

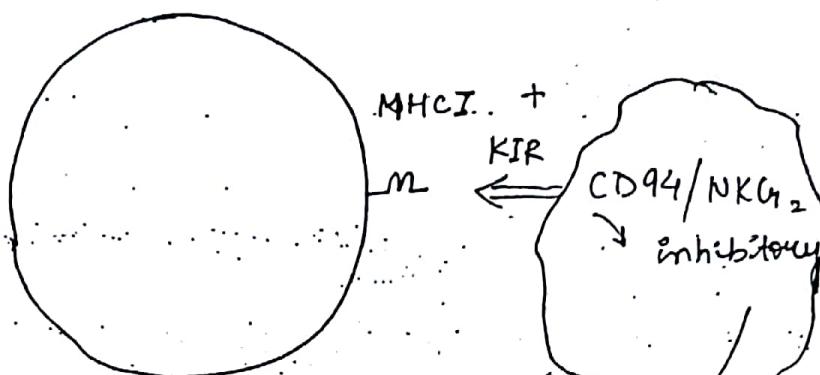
due to contral of vascular endothelial cell.

NK Cell

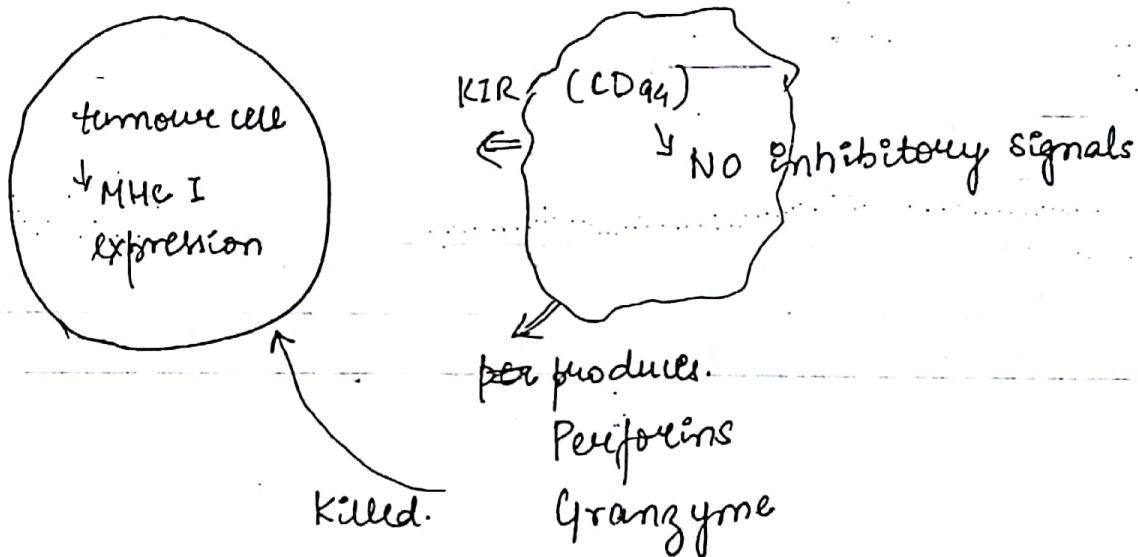
MHC-I in Exogenous Ag

69

Normal cell

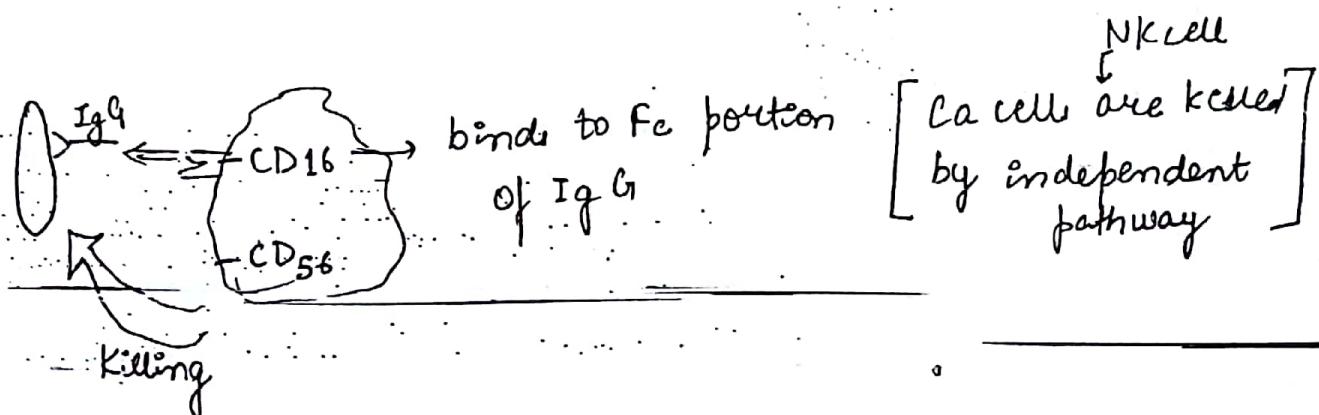


altered cell



Ab independent killing by NK cell.

NK cells → ADCC (Ab dependent cell mediated cytotoxicity)



DEFICIENCY OF PHAGOCYTOSIS

70

(I) Leucocyte Adhesion Deficiency (LAD)

Recurrent infections

No pus → as neutrophils can't come out

Omphalitis

Extreme neutrophilia ($> 30,000/\mu\text{L}$)

LAD I

→ mutation in β_2 integrin (CD18), gene (adhesion)

AR

LAD II

→ deficiency of fucosyl transferase [selectin]

↓
Molling (-)

LAD III

→ deficiency defect in regulatory protein kindlin

(femt³) activates ligand for β_2 integrin.

LAD > LAD III > LAD II

(II) G6PD Deficiency

Deficiency of enzymes in HMP shunt

Same as CGD (Chr Gr. Ds) → associated

Anaemia

III. MPO Deficiency
Granule enzyme Deficiency

71.

IV Chediak-Higashi Syndrome

AR

Defective Intracellular transport Protein (LYST).
Recurrent infec., chemotaxis factor, degranulation defect.

Absent NK cells

Partial albinism.

Δ - Giant Lysosome

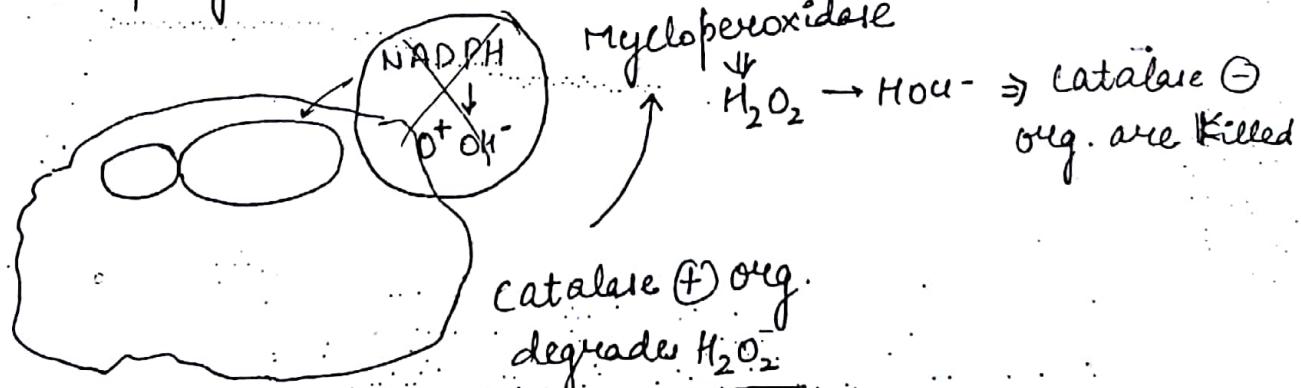
V Chr. Gr. Ds

- XL or AR

- Deficiency of NADPH

- Recurrent infection of \ominus Catalase +ve Bacteria

* Fungi



Candida

Aspergillus

Staph. aureus

Haemophilus Inf

Mtb

Enterobacteriaceae

① NBT (Nitroblue Tetrazolium) -

2) Neutrophil Oxidative Index

72

If agammaglobulinemia
recurrent catalase +ve infec'] \Rightarrow Bruton's
NBT (+)

B cell Deficiency

1) Bruton agammaglobulinemia

Deficiency of tyrosine kinase B cell maturation

XL

No Ig,

Prec B cells in bone marrow

marrow CMI

2) X Linked hyper IgM syndrome

- Deficiency of CD40L on activated Tcell

- Recurrent resp. + GI infec' [IgA is absent].
mucosal immunity

3) Selective IgA deficiency

IgA - M/c deficiency

Repeated sinopulmonary + GI infec'

No role of passive immunisation

Passive Immunisation
Deliver \Rightarrow IgG

4) Common Variable Hypoγ Globinemia

Onset - Late teens

73

B cell tnt in blood

↓ Ig over time

↑ autoimmunity

5) Transient & hypogammaglobulinemia of infancy

Delayed onset of normal IgG synthesis

Detected in 5th-6th months

Resolves by 2½ years

6) Job Syndrome [Hyper IgE syndrome]

Due to deficiency of T helper 17 ($\boxed{\text{Th17}}$) cell.

Retained 1° tooth

Cold abscess

Coarse facies

Eczema

$\boxed{\text{Th17 cell}}$ → encoded by Chromosome 17

Differentiated from TH0 cell by IL-6, TGFβ, IL-23

TH2 produces → IL-17, IL-22

IL17A IL17F



Stimulate B cells to produce

↑↑ Ab except IgE



- ① Prevent pyogenic fungal inf.
- ② Inflammation

- ③ autoimmunity
- ④ transplant rejection (ADcc) 74

p40 knock out Mice → Deficient Th₁₇
↓
↑ IgE

T cell Deficiency

- 1) Di George Syndrome
- 2) Bare Lymphocytic Syndrome (BLS) [Image]
Granulomatous necrotizing Lesion in mid face
- Type I → TAP Deficiency → MHC I ↓
- Type II → MHC II ↓

Combined partial T + B cell Deficiency

Wiskott Aldrich Syndrome

XL

Defect in cytoskeletal glycoprotein (WASP)

Ataxia Telangiectasia

Defect in kinase involved in cell cycle

Ataxia

Telangiectasia

Dif of IgA + IgE.

Complete Functional B & T cell Deficiency

75

SCID

• IL-2R γ or JAK 3 deficiency

[cytokine Receptor]
deficiency

Defective signals from IL4, 7, 9, 15, 21

XL, AR

• Adenosine Deaminase ADA or PNP (Purine nucleotide phosphorylase deficiency) toxic metabolite in T, B cell.

AR

• Zeta chain associated protein ZAP deficiency
defective signal from TCR

• Rag 1 or Rag 2 nonsense mutation. AR
No TCR or Ig gene rearrangement

Total absence of B & T cell

HYPERSensitivity

76

	Immune mediator	Immune Response	Response Time
Type I.	IgE.	Humoral.	2-30 min.
Type II	IgG > IgM	"	5-8 hr.
Type III	Immune complex (IgG)	"	2-8 hr.
Type IV	Tcell	Cell Mediated	24-72 hrs.

TYPE-I

→ 1° response to allergen.



Sensitisation of mast cell

→ IgE



→ 2° response to same allergen



Cross-linking



degranulation



Mediators of Type I HSN

1°

2°

77

Histamine, Heparin

PAF

Serotonin

Leukotriene (SRS-A)

Eosinophil chemotactic factor

PGI₂

Neutral chemotactic factor

Bradykinin

Proteases (tryptase
chymase)

Cytokines

IL: 1, 3, 4, 5, 6, 10, 13

TNF α

TGF β

GM-CSF

Eg.

- 1) Anaphylaxis
- 2) Atopy
- 3) Allergic rhinitis (Hay Fever)
- 4) Asthma
- 5) Food allergy
- 6) Allergic eczema

7) ABPA (Type I > Type II > Type IV)
[allergic bronchopulmonary aspergillosis]

Type - II

78

Autoimmune

Cytotoxic



- ADCC ↑
- complement mediated
- auto lytic

Non-cytotoxic

"cellular func" altered by
auto antibody.
eg. Gravé's Ds

e.g.,

- 1) Autoimmune haemolytic anaemia
- 2) Agranulocytosis, thrombocytopenia
- 3) ARF
- 4) Goodpasture Syndrome
- 5) Transfusion Reacⁿ
(ABO incompatibility)
- 6) Erythroblastosis fetala
(Rh incompatibility)
- 7) Drug induced haemolytic anaemia
[black water fever]
[penicillin therapy]
- 8) Myocarditis in Chaga Ds
(American Trypanosomiasis)
- 9) Hyperacute Graft Rejection

Immune complexes



causes tissue damage



extravasation of neutrophil



reach site of injury



focal area of granuloma

Eg's

1) SLE

2) Rheumatoid Arthritis [III > IV]

3) Polyarteritis Nodosa

4) Multiple Sclerosis

5) Serum Sickness

6) Arthus Reac"

7) PSGN

8) Lepros Reac" Type II

9) Meningitis

10) SABE

11) Hepatitis B + C (arthritis)

12) Mononucleosis

13) Dengue (arthritis)

14) 5th disease (step chick)15) Nephrotic syndrome in P. malarie.

16) Kala azar fever in schistosomiasis

17) African trypanosomiasis

18) Penicillin + Sulphonamide allergies

Type IV

80

Intracellular Pathogens

- 1) M. Leprae, M.Tb
- 2) Listeria monocytogenes
- 3) Bacillus abortus
- 4) Pneumocystis jirovecii
- 5) Candida albicans
- 6) Histoplasma capsulatum
- 7) Cryptococcus neoformans
- 8) Herpes simplex virus
- 9) Varicella
- 10) Measles

Skin Test

Tuberculin, Lepromin

Montenegro, Fice Test

Contact Dermatitis

Perylene chloride

Hair dye

Nickel salts

Poison

Ivy

Poison oak

Hashimoto's thyroiditis

Type I: Insulin Dependent DM

Gullain Barre

Celiac disease

Graft Rejection [IV > II]

Lepre Reac^b Type I.

Hypersensitivity pneumonia
[IV > III]

BACTERIOLOGY

81

Drug Resistance

Chromosomal

Meh

Mut) → Mutation in the chromosome
e.g. Mtb

→ Transduction

e.g. MRSA

Con

Plasmid

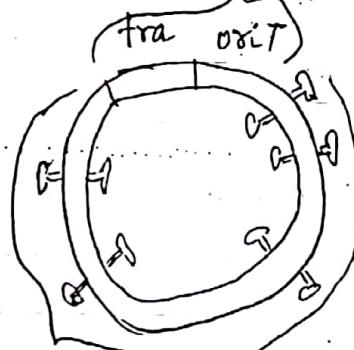
Mech

→ Insertion of Transposons

carrying blood resistance genes
in plasmid

Antibiotic Selection Pressure

RTF = resistance transfer factor.



Rd-resistance determinant

R plasmid = Rd + RTF

Combination = useful Rx.

Not useful
→ 8 Aug (R) seen.

Metabolic Defects

not seen.

(Plasmid doesn't code for metabolic)

e.g. INH \textcircled{R} Mtb \rightarrow
catalase \ominus
peroxidase Θ

Anti-microbial Susceptibility Testing

82

Mc Muller Hinton Agar

Middle Brook - M. tb.

Blood Agar - Haemophillus

Method

1) **Dilution Method**

Broth dilution

Agar dilution

* Broth Dilution method for MIC testing

Serial dilution of Ab



Std. inoculum

Bacteria: 0.5 McFarland MIC

Fungi: 2 Mc Farland



Density best measured by
Spectrophotometer

Min. Bactericidal Concentration (MBC) \Rightarrow estimated by Subculture

MBC \geq MIC

MEC - Min. Effective Concentration

Done for Anti-fungal Susceptibility

Min. concⁿ at which distortion of Hyphae is seen
morphologically

2) **Diffusion Method**

More common

a) STOKES meth [European]

b) Kirby bauer

(Disk Diffusion) [Image]

as per CLSI

(Clinical Laboratory Std. Inst.)

USA

c) E-test

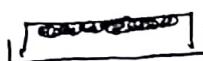
Disc Diffusion Test

83

- CLSI
- 9cm diameter Petri Plate
- 6 disc used each 6mm diameter.



Incubate



Haziness → resistance

Clear zone → inhibition.

MIC can't be assessed.

- E test (epsilometer test) [Image]

Diffusing Ab Gradient on a strip

✓ Diffusion technique in E MIC can be tested.



GRAM +ve Cocc

84

Staph
Catalase (+)

Grape like clusters

Strepto

(-)

chains

S. salivarius - longest

Gram +ve cocci

Catalase

(+)

Staphylococcus

(+)

S. aureus

S. intermedius

S. hyicus

coagulase

(-)

CONS

S. epidermidis

S. saprophyticus

Streptococcus

(-)

Gr A

Gr B

Enterococci

Bacitracin
Sensitive

CAMP

(+)

Pneumococci

Bile
Soluble

Bile
esculin
hydrolysis

STAPH AUREUS

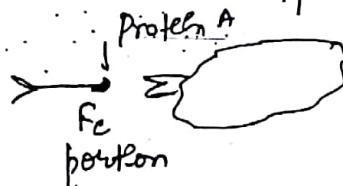
85

(I) Virulence Factors

↓ susceptibility to opsonisation

1) Protein A

- opsonin / Ab depletion by protein A
- binds to Fc portion of IgG thus preventing binding of Ab to macrophages



- 90% of Staph have protein A [COWAN I strain]

2) SCIN (Staphylococcal complement inhibitor protein)

- binds + inactivates the C3 convertase of alternate pathway

3) Protease /

Degradation of IgG, C3b by Staphylokinase

4) Clumping Factor - activated Factor H.

5) Extracellular Fibronectin Binding Lectin.

Inactivates C3 or prevent cleavage of C3.

6) Capsule

prevents binding of phagocytes to opsonins

Others

86

1) Peptidoglycan

2) Yichoic Acid

3) Toxins Mice of β haemolysis, \uparrow virulent

a) Hemolysin - $\underbrace{\alpha, \beta, \gamma, \delta}_{\beta \text{ hemolysis}}$

β toxin \rightarrow sphingomyelinase

\downarrow secreted

\downarrow virulent

γ toxin \rightarrow Panton Valentine leucocidin [PVL gene]

2 component toxin [S & F]

- C binds C γ toxin

synergohymenotropic toxin

(Q) [associated with community Acq MRSA]

b) Epidemolytic Toxin / Exfoliative Toxin -

Destroys mucopolysaccharide of Stratum Granulosum



BULLAE (Boil)



SSSS [Staph scalded skin syndrome] [Inogen]

children - Ritter's disease] fatal

Adult - TEN]

Pemphigus neonatorum] mild form
Bullous Impetigo]

Δ - toxin detection.

α Enterotoxin =

SuperAg M/c
cause TSS \downarrow by B, C + F.
Phage typing → Gen I.
8 types
A B C_{1 to 3} H I F Tampoons.

Δ - Nicolsky Sign +ve
desquamation on pressure

Food Poisoning

- Type A M/c

Due to preformed Toxin

IP < 6 hrs

vomiting (regal or stimulation)

'outbreak'

Δ - culture of vomitus / food.

4) Coagulase enzyme

↓
free.
enzyme

Bound.

clumping factor
Slide test

activate coagulase reacting
factor (CRF) in Rabbit / Human plasma

↓ used in lab

fibrinogen → Fibrin.

Tube coagulase Test [Image]

Tube coagulate

1:6 dilution plasma

88

Read at 4hr.

Released in log phase

5) Hyaluronidase ↑ virulent

If destroys tissues

(II)

M/c c of boils / abscess

osteomyelitis except

" Sickle cell anaemia → salmonella

" IV drug abuser → Pseudomonas

" " epidural abscess

" native valve endocarditis

" Hospital acquired Infection

(III) △

Culture on 5-10% Blood Agar

Selective media → Ludlam media

Salt milk Agar

Pigment → Nutrient Agar

(Golden Yellow) = glycerol mono acetate

Specific Test for S. Aureus = Mannitol Fermentation Test (+)

Phosphatase (+)

Heat stable nuclease (+)

S. intermedius

ZOONOTIC → Dog

89

Q In 2 wks, 5 newborns in the NICU developed *S. aureus* bacteremia. PFGE of the isolates were similar. Which of the following should be done next.

- Prophylaxis of all newborns in the NICU with vancomycin
- Protective isolation of all newborns
- Ensure strict hand hygiene
- Collection of nasal swab of health care worker.

Best Typing Method → PFGE (pulsed field gel electrophoresis) or sequence based typing.

Phage Typing [Image]

↳ Susceptibility to different phages among *S. aureus* strains

for virus = agarose gel electrophoresis preferred

Lysis count ⇒ If 1 bacteria infected = many phages.

(undergoing any replication of phage)



Uses :- 1> *S. aureus*

M/c phage type in India = Group II

Most useful in epidemiology

2> *Shigella*

3> *Vibrio*

M/c = editor

4> *Salmonella*

M/c - E₁

Least useful in epidemiologically

Vi phages used in Salmonella

90

Vi Ag = S. Typhi

S. Paratyphi

S. Dublin

Citrobacter

Father of Hand Hygiene = Edward Semmelweis
obstetrician
5th of May - Hand Hygiene Day

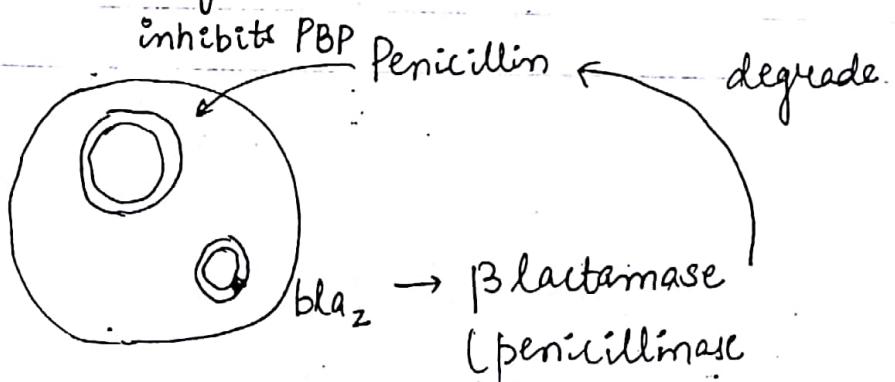
5 moments

Before & after seeing apt

Before & after a procedure

Contact ∞ pts. surrounding

IV Drug Resistance



- Plasmid encoded Drug R → By Transformation
- By Transduction - 90%
- Conjugation - 10%

Penicilline Resistant Penicillin \Rightarrow \ominus PBP \rightarrow Degraded Penicillinase

Methicillin

Dihydroxyacetone

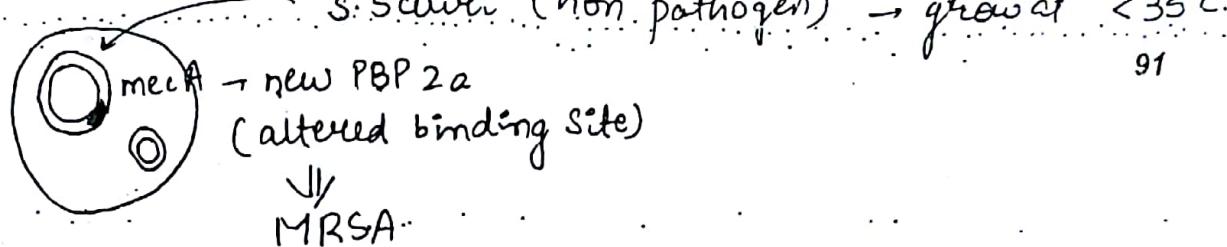
Oxaillin

Carbenicillin

Cloxacillin

Nafcillin

Transduction



91

MRSA

Hospital Acquired Infec"

Chromosomal

DOC → for t/tng MRSA → Vancomycin

Screening → Nasal swab (50% colonisation)

Hand 40% colonisation



Mannitol Salt Agar + Cefoxitin

↓
yellow colonies

30°C → incubation [me_cA gene expression
best at 30°C]

TBC media for Vibrio.

Green coloured due to citrate

Q. 25 yr old Girl presents to OPD with carbuncle at the back of neck. Pus aspirated reveal MRSA is true about the strain:

a) ↑ Resistance

b) ↓ Virulence

c) Associated with SCC_{mec} I II III [Staphylococcal cassette chromosome → pathogenicity island]

d) " " ~~pvl~~

Criteria of HAI :-

After 48 hrs of admission

or

in 2 weeks of Discharge from hospital

or

in ~~12 weeks~~ month of Discharge in case of prosthetic implant

HA-MRSA

↓ virulent

CA-MRSA

new strain

↑ virulent

SCC_{Mec} I II III

SCC_{Mec} **IV** V VI

prl associated

↑ Resistance

↓ Resistance

↓

Clindamycin (R)

Clindamycin susceptible

D test (+

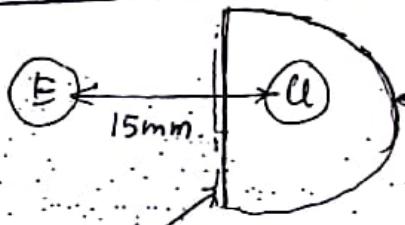
(-)

D Test

To detect **Inducible** Clindamycin Resistant Strains expressing Erm gene

↓
Erythromycin → induces Clindamycin (R)

This can't be done by Diffusion Test



as susceptible strain
grows away as resistant
is not developed this side

Virulent strain

due to erythromycin grows
towards clindamycin

VRSA Plasmid encoded

vanA gene derived from [Enterococcus]

$\geq 16 \mu\text{g/mL}$

↓ common.

93

VISA (Vancomycin Intermediate \textcircled{R} Staph. aureus)

↑ cell wall synthesis

$4-8 \mu\text{g/mL}$

VSSA (Vanco Susceptible S.A.)

$\leq 2 \mu\text{g/mL}$

MIC → Done for VRSA

— Neisseriae

Done by Broth Dilution

Q. Accessory Gene Regulator (ag^4)

MSCRAMM (Microbial surface component Recognising
Q [adhesion] matrix molecules)



Protein A ; Clumping Factor, Lectin.

Modified Hodge Test
for carbapenemase \textcircled{R}

(+) S. Epidermidis

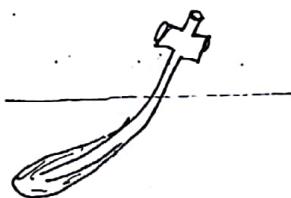
75% infec.

Skin commensal → Lysostaphin

↓
(-) S. aureus

R/F - IVC (central line)

Intercellular adhesin → adhere to cellular tip.



↓
thin polysaccharide
BIOFILM (300 nm)

↳ anti-phagocytosis

Antibiotic (R)

C/F

A M/c cause of prosthetic valve endocarditis

(early onset) - \leq 12 months - HAI

Late onset - Streett. Viridans

M/c of CR BSI (catheter related Blood Stream Infection)

Δ of CRBSI → culture of catheter tip (5cm)

Congo Red stain → biofilm.

Confocal microscope

↓
multiple focal point image

Superimposed

In situ Δ of CRBSI → BACTEC blood culture of 2

- Samples

- Catheter

- peripheral vein

If $> 2 \text{ hr}$ difference in positivity \rightarrow CRBSI

Differential to time test positivity

~~if < 2 hr~~

↓
Btw the +ve of 2 samples

Catheter \rightarrow Peripheral vein.

95

(II) *S. Saprophyticus*

UTI

Novobiocin (R)

STREPTOCOCCUS

96

α hemolytic

Pneumococcus

S. viridans Gr.

S. mutans

(dental caries)

β hemolytic

Lancefield or

C-Carbohydrate

A to V except I + J

γ hemolytic

Grk A - Pathogen

Griffith Serotyping

based on M protein

Gr. D - M/c - γ hemolysin

Intestine

Enterococci

{ S. faecalis H/c. }
S. faecium Most R } They Don't Grow in PYR(+) 6.5% NaCl.

S. Bovis (S. Gallolyticus) → Grow in 6.5% Bile NaCl
↳ α /c Ca Colon. PYR(—)

GRP-A STREPTO

Virulence Factors

1) Pyrogenic Exotoxin (cytotoxigenic)

Scarlet Fever → Rash (pastia's line)

Sandpaper appearance

Strawberry tongue

H₂₈ - H/c serotype

2) M Protein

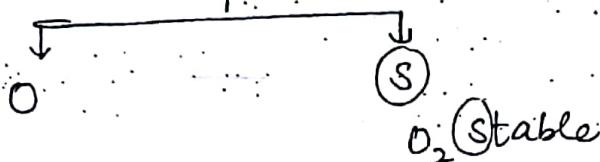
antiphagocytosis
Serotyping



97

T + R protein \Rightarrow No virulence.

3) Hemolysin (Streptolysin)



O_2 labile
active in reduced or anaerobic state

ASO titre

ARF $>$ 200 IU

seen in PSGN & Pyoderma

Tonsillitis strains M_{1, 5, 12, 24}.

Molecular mimicry \rightarrow Ab against cell wall protein of GrA strept. cross reacts with myocardium.

4) Streptodornase

destroys DNA

Anti-streptodornase B \Rightarrow Marker for PSGN
Pyoderma

↓
Skin pathogens

M_{25, 43, 53-55, 59-61}

5)

Streptokinase

dissolve clot

used as thrombolytic agent → source is Grp C or *S. equisimilis*

98

6) Hyaluronic acid in capsule

7) Hyaluronidase → destroys tissue

Flesh eating bacteria

Grp A Strepto M₁ to 3

↓
Necrotising Fascitis

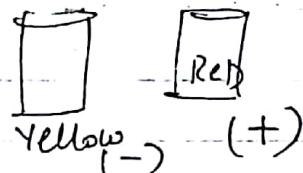
Diagnosis

PYR (+)

Bacitracin sensitive.

PYR Test

Aminopeptidase enzyme



Releases free β naphthylamide

pyrrolidonyl β naphthylamide

PYR

(+) Grp A Strept. Enterococcus

(-) Grp B Strept, bovis

G/R. B. STREPTO. [S. GALACTOSAEMIA]

99

↳ Bovine mastitis

20-40% female → genital tract

↓
Neonatal Meningitis (direct spread)

M/c

Δ - Hippurate hydrolysis +

CAMP + IOC

Hippurate Hydrolysis Test

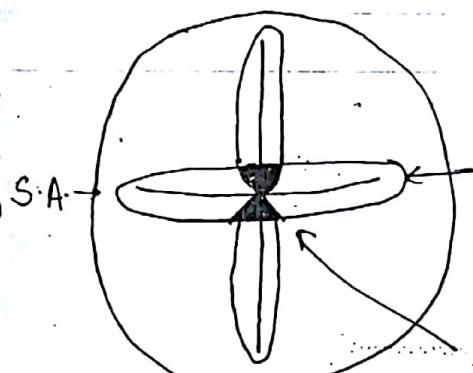
[Image].

Glycine + ninhydrine purple colour.

Hippurate enzyme acts on Hippurate

CAMP

G/R B strepto → stimulate Staph
to release β toxin



Butterfly hemolysis

β hemolytic
strepto

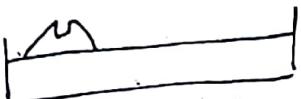
PNEUMOCOCCUS

100

Lanceolate shaped diplococci \pm capsule



Draughtsman colony (cavum coin)



Virulence Factor

- 1) capsule
- 2) Ig A₁ protease
- 3) Pneumolysin } Not secreted
- 4) α toxin

Autolysin \rightarrow destroys bacteria
to release

C/F

- M/cc of pyogenic meningitis
- " " community acquired pneumonia
- " " Hosp. acq. pneumonia (VAP)
except

after 5-6 days } Pseudomonas.
MDR strain }

Δ- Otitis media in <5yr

① Inulin Fermentation +

② optochin ③ \rightarrow Screening test

③ Bile Solubility \rightarrow confirmatory

R

Meningitis → start w/ ceftmoxone + vanco

101

↓
Penicillin

other infec' → start w/ Penicillin

↓
ceftmoxone + vanco

Vaccine

Adult → PPSV (polyvalent polysaccharide vaccine)
23 seroprevalent strain

High Risk

- splenectomy
- cochlear implant
- >65yr
- ch. lung, kidney, liver, heart Ps
- immunocompromised.
- Diabetics
- Hospitalised pts w/ H/o smoking alcohol.

child - PCV13 [Pneumococcal Vaccine]

2 - 24 months

OSLER TRIADS / AUSTRIAN SYNDROME

Pneumococcal endocarditis (Aortic valve) + Meningitis + Pneumonia

ENTEROCOCCUS

102

↑ Resistant → UTI, HAI

↳ (R) to penicillin - hyperprod⁺ of PBP₅

6.5% NaCl

9.6 pH

46°C

40% Bile

Δ → PYR (⊕)

Bile esculin hydrolysis (⊕)

S. Bovis → PYR (⊖)
[Galactosidase] ⇌ Bile esculin Hydrolysis (⊕)

~~VRE~~

VRE

plasmid

VAN A (MC) → (R) to both vancomycin + Teicoplanin

Van B, C, E → (R) to vanco only

Substitution of terminal

D alanine = D serine/
Lactate

↓
Elimination of Target

HLAR → high level aminoglycoside (R)

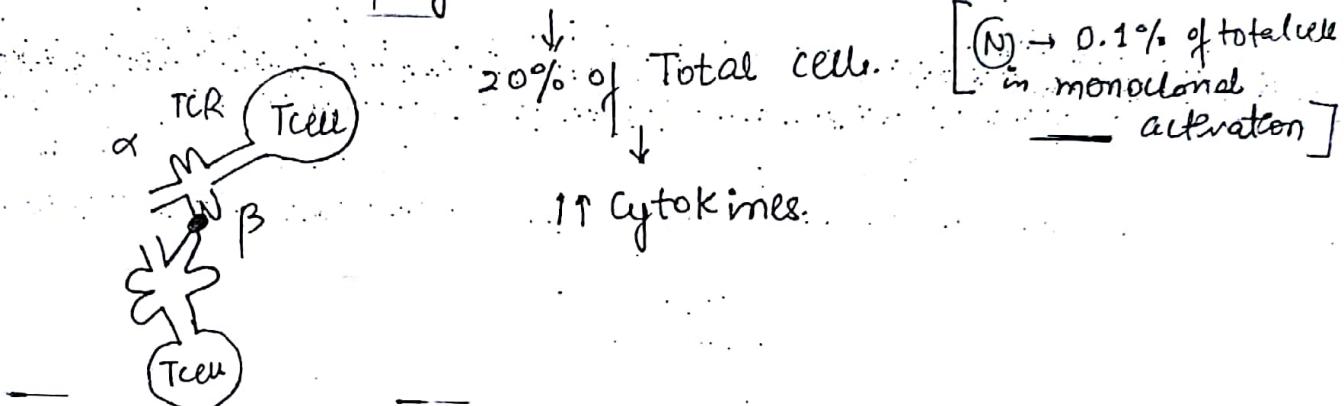
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SUPER ANTIGEN

MHC unrestricted

Bind to $\text{V}\beta$ region of TCR

polyclonal activation



Pathogen showing superantigen

- 1) Staph aureus
- 2) G+ A strepto
- 3) M:tb. including BCG
- 4) Rabies
- 5) HIV
- 6) EBV

IgA → Mucosal Affinity

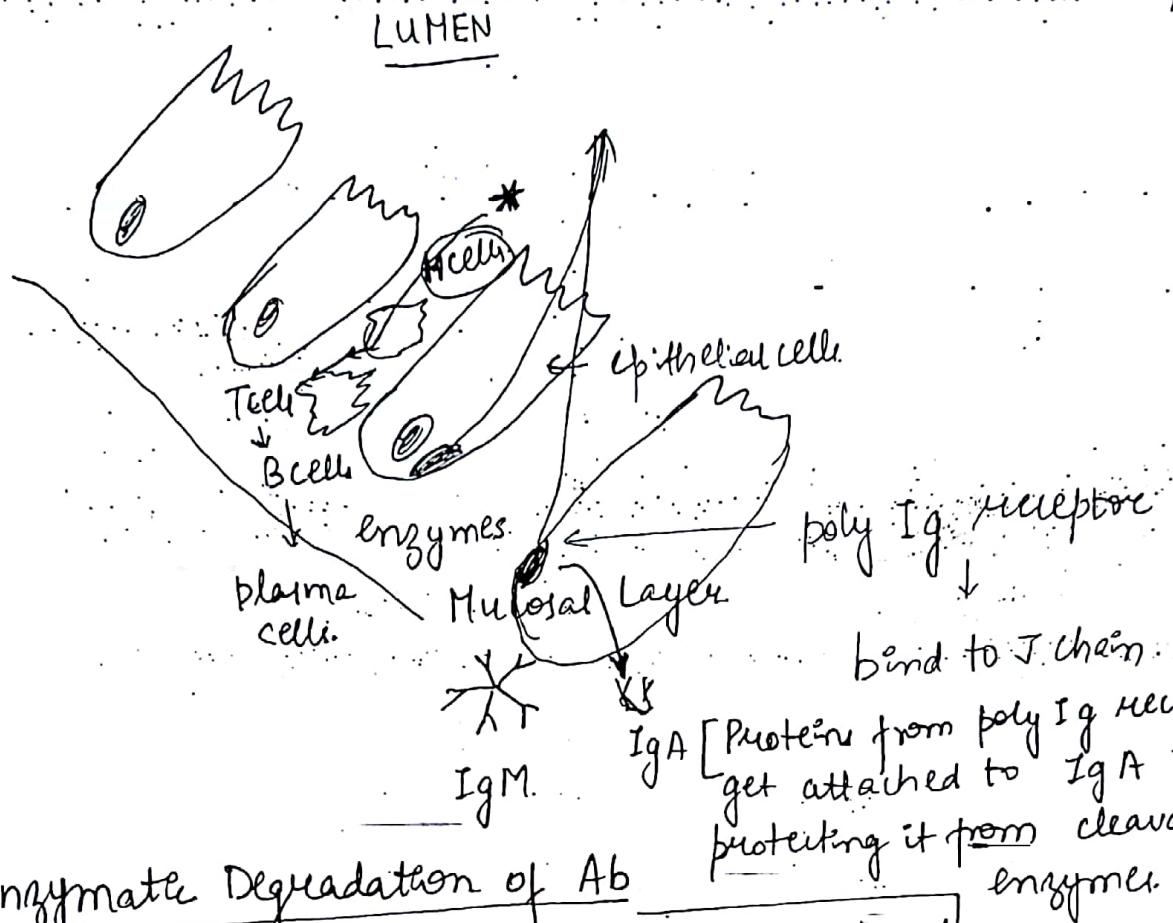
↪ Ig doesn't cross placenta

a) IgG₁

b) IgG₂ → placenta doesn't have receptor for Fc portion of IgG₂

c) IgG₃

d) IgG₄ [Receptor mediated Trans cyto of Ab]

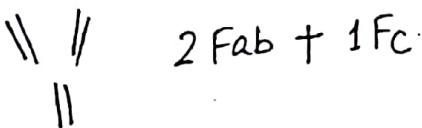


Enzymatic Degradation of Ab

Pepsin → cleaves below di-sulphide Bond.

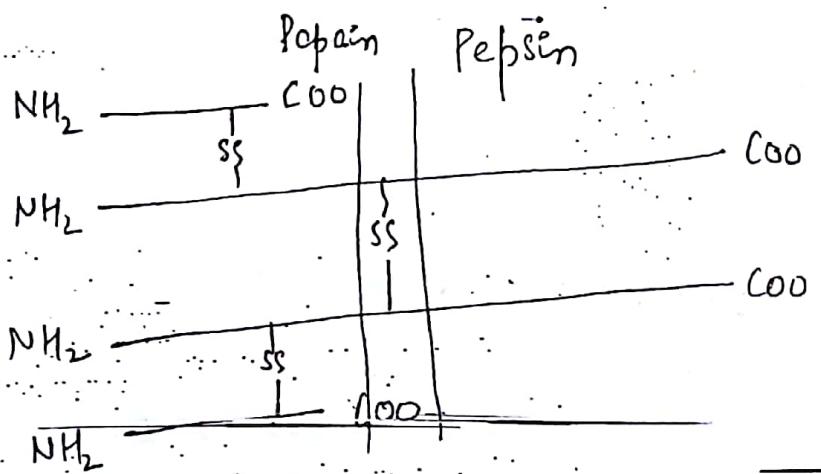


Papain → cleaves above disulphide Bond



[secretory portion]

[↓ derived from part of poly Ig receptor.]



H chain is cleaved → Isotype func" is lost

105

L chain is intact → Idiotype intact

Ag. Binding occurs.

Valency (monomer)

Pepsin → unchanged

Pepsin → 2 → 1

2ME - 2 Mercaptoethanol → cleaves disulphide bond

2H + 2L chains

Idiotype, Isotype all lost

DIFFERENTIATE *S. AUREUS* FROM *MICROCOCCUS*

Micrococcus

Obligate aerobe

Non-pathogen

Tetrad

Skin commensal

Hugh & Leitson's oxidation Fermentation Test

Fermentation

S. aureus

Oxidation

Micrococcus

Disc in Blood Agar

Group A strepto → Bacitracin (S)

Pneumococci → Oftacrin (S)

* Non-Streptococcal catalase -ve Gram +ve cocci 106 24/2/18

→ Pediococcus * Leuconostoc → Vancomycin Resistant

→ Abiotrophie + Granulicatella species

nutritionally variant streptococci → require Vit B6

DOC → Gentamicin + Penicillin (to avoid resistance)

* S. Angiosue Gr

Agglutinate A, C, G, F antiserum

VP test +

Butterscotch, or caramel odour.

GRAM +ve BACILLI

I LISTERIA MONOCYTOGENES

1) Only Gram +ve Bacillus & has Endotoxin.

2) Intracellular pathogen.

[Int. A & B toxin] → helps in internalization.

3) Listeriolysin O secreted by Listeria destroys phagosome
(LLO) & thus escape phagocytosis.

Listeriolysin, cytotoxin ⇒ helps in escaping phagocytosis

→ Hemolysin

shows β hemolysis

3) Actin Filaments

help in intracellular & intercellular motility



BLEB Formation

[Image]

4) Cold Growth. (2-8°C)

5)

5) C/F → Neonatal Meningitis

Early Onset

Granulomatous infantiseptica

Intracellular Transmission.

Mother is asymptomatic

Disseminated. Cold "

Late Onset

10-30 days after birth

Neonatal Meningitis

Mother is asymptomatic

Spread through fecal contamination by health care workers.

Adult ⇒ Food Poisoning

By consumption of Refrigerated food.

6) A → ① Blood Agar → β hemolysis

② CAMP test → (+)

③ Anton test → (+)

④ Gumbling Motility at 21°C.

⑤ Ab detection

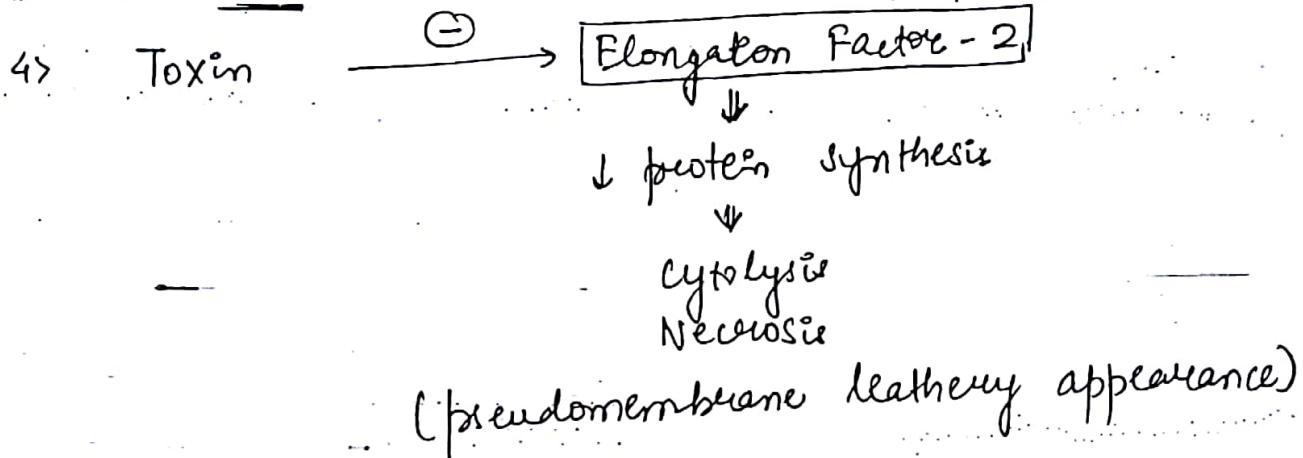
⑥ PCR (↑ sensitivity) - Good method nowadays.

(II) CORYNEBACTERIUM DIPHTHERIAE

108

- 1) Gram +ve Bacilli
- 2) Metachromatic Granules are not seen in Gram +ve
- 3) For Pathogenesis, toxin is important

↓
It depends on Iron Conc.
[0.1 mg/lc]



5) △

- a) Culture → Loeffler's Serum Slope (in 4-6 hrs)
 ↑ patient.
- Tellurite [carrier → selective media]
 preferred for carrier state
- If not specified patient or carrier → go for LSS

→ Hoxley, Tinsdale
Miss Serum Water

b) Specimen → Throat Swab

GRAVIS

INTERMEDIA

~~Poached Egg Colony~~
MITSIS

Dairy Head
Colony

Frog Egg colony

Poached Egg Colony

More virulent

c) Microscopic "exam" of Throat Swab
for toxigenicity.

i) Albert Stain

Metachromatic

ii) Neisser Stain

or

iii) Pander

Volutin

or

Baber Ernest granules.

↓
Pathogenic.

d) Toxigenicity

In Vito → Guinea Pig

(250 - 350 gm)

Subcutaneous

Intercutaneous

S/c inj of test strain

death in 96 hrs

Use control → S/c inj of test strain + 500 IU of diphtheria antitoxin intraperitoneally

Protective dose

4 Guinea Pig → 3 for test + 1 control used

Advantage - 1 test performed on 1 guinea pig

1 IU of Antitoxin = Min. amount Required to neutralise 100 µg of toxin.

Rabbit - 500 gm
Guinea Pig - 250 gm
Mice - 20 gm

IV inj of test strain
↓ after 4 hrs
50 IU of diphtheria antitoxin intraperitoneally

Cutaneous Lesions in 48 - 72 hrs.

Website: http://mbbshelp.com

WhatsApp: http://mbbshelp.com/whatsapp

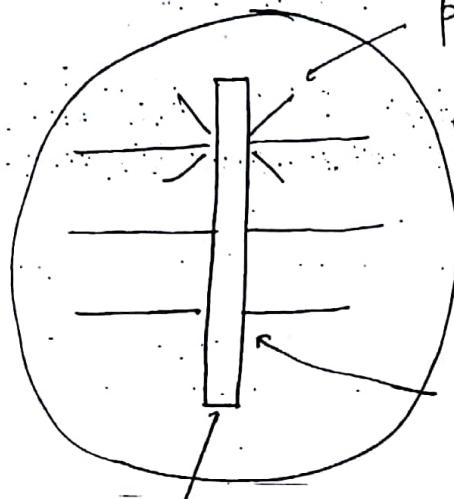
In Vitro → Elek's Gel precipitation

110

Double Diffusion in 2 directions
[Ouchterlony Procedure]

Horse Serum Agar

ppt. Line due to "heat"



diphtheria antitoxin
1000 IU/mL

(+) control

test strain

(-) control

No line of
ppt.

If line (+) → Toxin produced

If line (-) → Toxin not produced

Corynebacterium Jeikeium ⇒ MDR

Urealyticum ⇒ Urease +ve (UTI)

Aerococcus haemolyticum ⇒ Reverse CAMP test (+)

SCHICK TEST (Neutralization Test)

for susceptibility

Toxin

(one arm)

Heat inactivated toxin

(other arm)

Positive (susceptible) (+)

(-)

(+)

(-)

Negative (Immune)

(+)

Pseudoreaction [immune but Hypersensitive] (-)

Combined: Reac⁺

(susceptible + Hypersensitive)

(+)

(+)

111

Toxoid is safe than heat inactivated Toxin.

So, no fear of anaphylaxis

DIPHTHERIA TOXOID

5-25Lf units/dose [0.1mL]

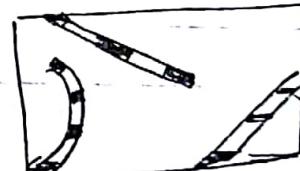
[Lf = Limulus
floculation]

TT → 10Lf

dT → low dose → 1-2Lf

↓
used in adults.

BACILLUS ANTHRACIS



Zoonotic Disease

Non-Motile.

Seasonal outbreak - summer season.

Dry Grass ← feeding Cattle



gets injured
↓

(spores + nt in soil) Spores enter
↓

Haemorrhage
↓

Death.

	INCIDENCE	MORTALITY
Cutanous Anthrax	95%	20% 112
Hide Porter Disease		
↓		
Malignant pustule		
↓		
Black Eschar		
Pulmonary Anthrax	5%	95%
Wool Sorter		
Intestinal Anthrax	Hare	95%

Virulence Factor

- 1) Capsule }
- 2) Toxin }
- 3) → Oedema factor (\uparrow cAMP)
- Protective factor } cytolysis
- Lethal factor

Plasmid

Diagnosis

Transportation of sample \Rightarrow Triple Layer packaging
(used in infectious samples)

Culture on PLET Media

[Polymyxin, Lysozyme, EDTA, Thallus acetate]



Medusa Head Colony \rightarrow

String of Pearl \rightarrow ○○○○○○

Bamboo stick or Box Car Appearance

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B. anthracis
(obligate aerobe)

also seen in *Clostridium Perfringens*
(obligate anaerobe)

Inverted Fir Tree → on Gelatin Stab culture



Mc Fadyen Reag" → for capsule

Confirmation

1) R. phase lysis

or

2) MLST (multilocus sequence typing)

— by PCR

[to distinguish other spore bearing, aerobic organism]

Bioterrorism → Category 'A' agent

	Most potent
<i>B. anthracis</i>	
<i>Versinia Berto</i>	
<i>Clostridium Botulinum</i>	
<i>Francicella tularensis</i>	
Small Pox	→ epidemiologically important.
Agent of Viral Haemorrhage fever	

epidemiologically important.

B. CEREUS

Motile

- causes food poisoning → By RICE

Food poisoning

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Emetic

vomiting

preformed toxin

< 6 hrs IP

↑↑ bacilli in stool

Enteric

diarrhoea

> 12 hrs IP.

↓↓ bacilli in stool

A - Culture - MYPA media
(mannitol Yolk Phenol Red novobiocin agar)

ACTINOMYCES



Gram +ve Bacilli [Image]

Filamentous

Obligate Anaerobes

A → Actinomyces

B → Bacteroides [Gram-ve]

C → Clostridium

No SOD Superoxide Dismutase

A No Permase

actinomyces.

Gram +ve Branching filamentous rods

Nocardia

Actinomycete

Anaerobe
Non Acid Fast
Endogenous Infe"
↳ Oral cavity
Lumpy Jaw - M/c

GIT
Genitourinary Tract
Thoracic Actinomycetes
↳ aspiration of oral Secretions

A of Actinomycetes → Actinomycetes involvement of pelvis occurs most commonly in association with an IUD.

↳ Sulphur Granules

MYCETOMA

swelling

sinus

granules

Nocardia

115

Aerobe
Acid Fast (10% H₂SO₄)
Exogenous Infe"

Nocardiae	Mycobacteria
entangled red bacilli	Long, slender Beaded

Actinomycetoma → actinomycete, Nocardiae + streptococcus

Eumycetoma → Fungi pigmented

Botryomycosis → S. aureus - M/c

Actinomycetoma

acute inflammatory cond'

Purulent D/c

white or yellow sulphur granules
except actinomadura fellitelli
↳ Red granules

Eumycetoma

Chronic

~~Purulent D/c~~

Serous D/c

Brown, Black stain.

Gram stain.

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2) CULTURE → Molar tooth Colony
Spidery colonies

Δ of NOCARDIA

1^o infection in Lungs → Brain + Kidney Abscess.
Subcutaneous Infec → Mycetoma

- Gram Stain

- Acid Fastness

- Culture using Paraffin Bait Technique

BACTEROIDES FRAGILIS [Gram-ve
Anaerobic]

1) Virulence Factors

Capsular polysaccharide

Protease, neuramidase

Cytolysin

Enterotoxin

LPS - 1000 times less Biologically potent

Drug resistance to β lactamase

2) LEMIERRE'S SYNDROME

Jugular thrombophlebitis

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Fusobacterium necrophorum → Bacteroid species

Lung emboli, sepsis

3) △ → culture Media

- a) Trypticase soy Agar
- b) Schaedler's blood agar
- c) BHI (Brain Heart Infusion)

→ + Kanamycin.

CLOSTRIDIUM

Obligate anaerobe

No O_2 / ↓ Eh (Redox Potential)

C. TETANI

Brewer stick appearance

Virulence factor -

Tetanolysin

Tetanospasmin

No virulence

plays role in virulence

② GABA + glycine

↓

② of presynaptic receptors

Spasmodic

Spasmodic paralysis

A - Robertson Cooked Meat Broth

1.18

Thioglycollate Broth



Blood Agar



Macintosh Field.

→ [Image].

(Molybdenum catalyst)

candle jar
↓
for capnophiles

BI for checking O_2 free environment

in Macintosh Field → Pseudomonas

↓
it does not grow if completely O_2 free

GAS PAK JAR

→ Better, safe

Prevention : Active Immunisation + Passive immunisation.

PEP for tetanus

Immunity Category

Simple wound

Contaminated wound

Cat A

Nothing Reqd

Nothing Reqd

Cat B

Toxoid 1 dose

Toxoid 1 dose

Cat C

Toxoid 1 dose

Toxoid 1 dose + HTIG

Cat D

Toxoid complete dose

Toxoid complete dose + HTIG.

A - taken complete course of TT / Booster in part [5 yrs]
in part 5-10 yrs

B - " " " " " in part > 10 yrs

C - " " " " " in part > 10 yrs

D - Not taken complete course of TT / booster in part 5 yrs.

Simple wound - < 6 hrs, clean, non-penetrating, negligible tissue damage.

Booster recommended every 10 years.

1° Immunization - 1st 3 doses on 6, 10, 14 weeks.
Safe vaccine.

Pertussis vaccine - cause fever as cellular component is used.

C. PERFRINGENS / WELCHI

12 toxins

α toxin → Most virulent

↓

decatheinzase c/ phospholipase

C/F

① Gas Gangrene

C. perfringens 60%

C. novyi / septicum 40%

② food poisoning

Type A

World wide

Necrotizing enterocolitis

α toxin → ileum → Necrosis + Gas

Type C

Enteritis

endemic

Otoxin

→ jejunum → Neurosis + Gas

Drambrand.

Germany

Pigbel

Papua New Guinea

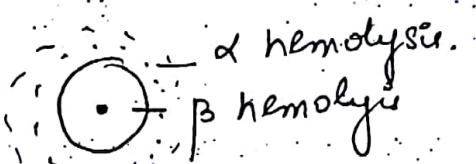
Neurotoxins

Poise In The Belly.

1 - ① Target Sign.

around clostridium \Rightarrow zone of β hemolysis due to θ toxin.

zone of α hemolysis due to α toxin



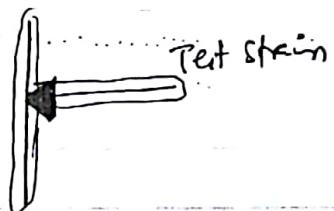
② Reverse CAMP Test (+ve)

- Group B streptococcus used ~~for~~
- * Test strain is inoculated.

Arrow type
hemolysis.

Bow Type hemolysis

Toxin of clostridium goes to
strepto + causes more
hemolysis.

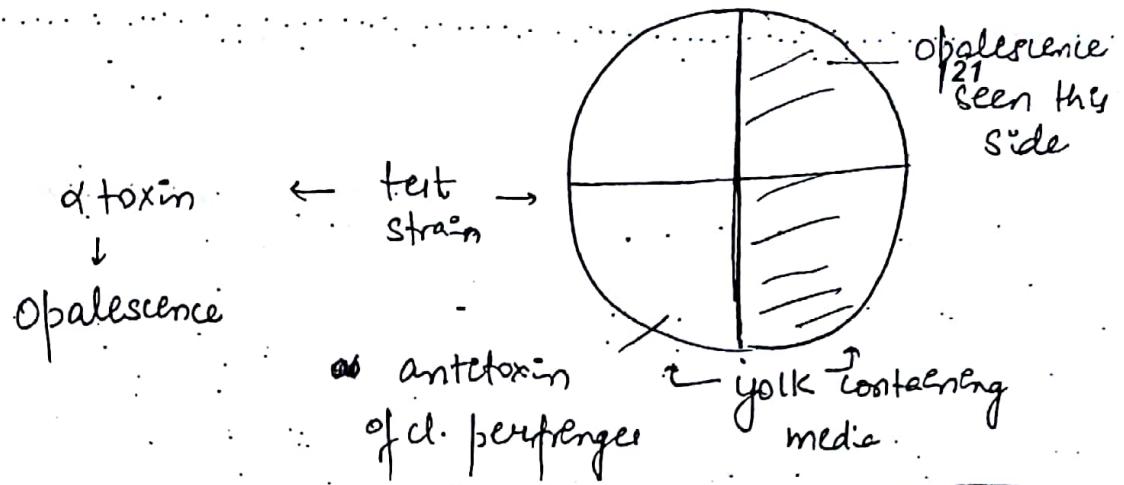


Toxin of strept. goes towards clostridium.
causes more hemolysis

③ Stormy fermentation on litmus milk

④ Nagler Test

Volk containing media



C. BOTULINUM

* Virulence Factor

Toxins → all are neurotoxins except C₂ (enterotoxin)

Botox Injecⁿ → anti-aging

Bioterrorism

* Pathogenicity -

1) (-) Ach → Flaccid Paralysis.

2) food poisoning

Preformed toxin in home made canned food.

IP - [8 to 36 hrs] Q.

3) Floppy child syndrome

↓ Spores (HONEY)

Infant gut microbiota starts developing in 4 hrs.

4) Wound Botulism

due to spores.

Δ - Toxin Detection

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C. DIFFICILE

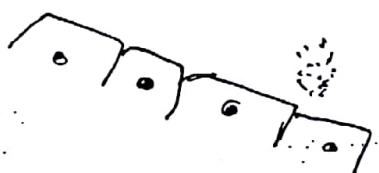
Causes pseudomembranous colitis due to Clindamycin
cephalosporin [2nd & 3rd generation]

* Virulence factors

Toxin $\begin{cases} A - \text{enterotoxin} \\ B - \text{cytotoxin} \end{cases}$ Both toxin glycosylate GTP binding protein of Rho subfamily
 \downarrow protein synthesis

* Pathogenicity -

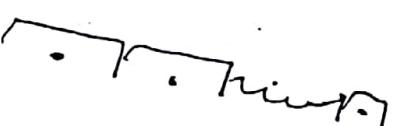
Scallop Lesions



Pseudomembrane



Necrosis.



No Gross Blood in stool.

* Δ

Test	Sensitivity	Specificity
Stool culture Best test for confirmation.	+++	++ ¹²³
Cell culture cytotoxin test on stool	++	(+++)
ELISA for toxin A & B in Stool	++	++
ELISA for c difficile common Ag in stool. Best Test	+++	++
NAAT for toxin A & B gene in stool	(+++)	(+++)
Colonoscopy or sigmoidoscopy	+	+++

Rx ① DOC - Metronidazole
or

Vancomycin. → ↑ effective → used in severe
causes.
To prevent incidence of VRE.

② Fecal Transplantation

GRAM -ve Cocci

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NEISSERIA

Intracellular, diplococci

Oxidase $(+)$

Media req. for growth = chocolate agar

Thayer Martin

New York City

Martin Lewis

Candle Jar useful \rightarrow as they are capnophilic

Oxidase Test

Kovac Reagent [P-phenylenediamine. H₂dihydrochloride]

↓
Cytochrome Oxidase

Blue (indophenol)

* $(-)$ \rightarrow Enterobacteriaceae

$(+)$ \rightarrow Pseudomonas.

Aeromonas

Neisseria

Campylobacter

Hemophilus.

N. MENINGITIDIS

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Virulence factor

- 1) Capsule
- 2) Endotoxin
- 3) ~~Bsp~~ Pili

C/F \Rightarrow Pyogenic Meningitis & Rash.
leads to outbreak.

Waterhouse Friedrichson Syndrome [due to endotoxin]

Serotypes A, B, C \rightarrow outbreak

Y, W₁₃₅ \rightarrow sporadic cases

X, Y, Z9E \rightarrow in HIV pts.

other \rightarrow carriers

Nasopharyngeal 5-10%

during outbreak 80-90%.

Chemoprophylaxis - 1g " ceftriaxone > cipro > Rifampicin.

Affects European countries. In India - Meghalaya

\downarrow
we use cipro

Vaccine - polyvalent polysaccharide vaccine

A, C, Y, W₁₃₅

(Ur B) \rightarrow Infect count deficiency of Late complement
proteins probrin

\rightarrow H/C infec in infants

\rightarrow capsule is non-immunogenic

Group B vaccine - based on subcapsular antigens.

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out in cell wall. { outer membrane vesicle.

Numerical adhesion factor A.

Factor H binding protein.

numerical heparin binding antigen.

* Rapid Carbohydrate Utilization Test

[ONPG test] [O-Nitrophenyl β -D-galactopyranoside].

{ β -galactosidase

galactose + O-Nitrophenyl-

{ [yellow].

N. lactamica \rightarrow ferments lactose unlike other N. species

N. meningitidis \rightarrow Maltose fermentation.

N. flavescens \rightarrow F, Sucrose "

N. gonorrhoeae \rightarrow glucose "

MORAXELLA CATARRHALIS

Cause - Otitis Media

Sinusitis.

COPD \rightarrow AE

A -- Hockey Puck Sign

colonies can be slid across the agar surface.

→ out disruption

→ Lacks carbohydrate fermentation used to differentiate from saprophytic

→ Produce DNAase Butyrate esterase. Nucleic

Drug Resistance to β -lactamase-

N. GONORRHOEA

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Virulence Factors

- 1) Pili Adhesion.
- 2) Opa (Protein II) - adhesion
 - transient ↑ in CDG
- 3) Protein (protein I)
 - endocytosis + invasion
 - \ominus Complements
- 4) Rmp (protein III) - Blocks Ab block effect of bactericidal
 - Ab to fowlin + LOS leading to reinfection
(lipooligosaccharide)
- 5) Ig A1 protease
- Fbp (Ferric Binding protein).
- Lip (HB)
- 6) LOS - endotoxicity
 - Chronic Infection.

Rept Pili, Opa, LOS express Phenotypic variation
Pair of different strains express different Ag typing

Proteins inside human body doesn't change → they remain same.
Lgt shows variation in different strains.

Resistance

PPNG - β -lactamase
 (plasmid)

Common

producing strain of N. gonorrhoeae
encoded

CMRNG - [chromosomal] encoded high level resistance
to penicillin. 128

Mare

TRNCH - High Level [Tetracycline] Resistance ($\text{MIC} \geq 16 \text{ mg/L}$)
- tet M gene on conjugative [plasmid.]

[Ceftriaxone] resistant - [chromosomally] encoded pen A gene
expressing PBP 2a.

Gonococcus doesn't affect Vagina

I.P. - 3-4 days. [chlamydia - 7 day]
H/C

Chlamydia diagnosed
by NAAT

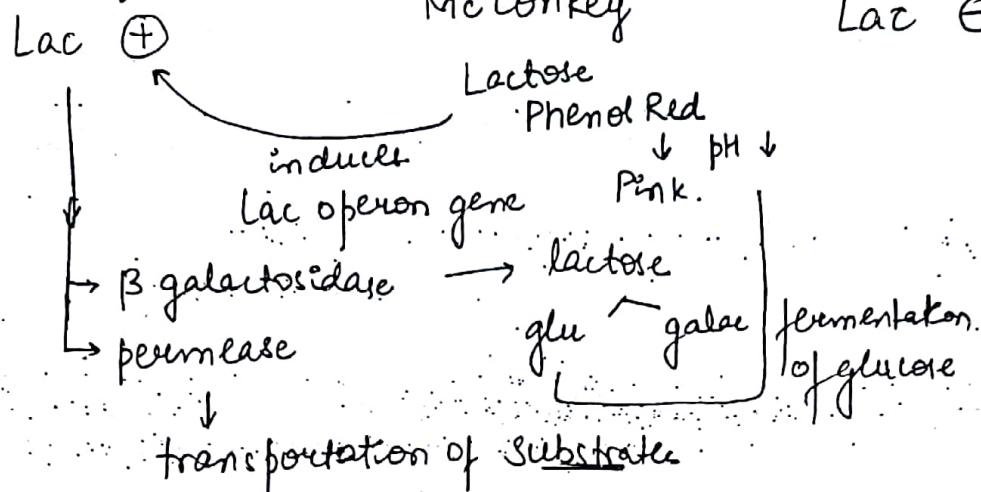
GRAM -ve BACILLI

ENTEROBACTERIACEAE

- ① Ferment Glucose
- ② Motile / Non-Motile
↓
peritrichous flagella
- ③ Catalase + except S. dysenteriae Type I
- ④ Oxidase -
- ⑤ Reduce nitrate to Nitrite

Enterobacteriaceae

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E. coli - [except. *Enteroinvasive E. coli*]

Salmonella

Klebsiella

Shigella

Enterobacter

Proteus

Serratia

Yersinia

Edwardsiella

Enteroinvasive E. coli

Citrobacter

Arizona

Providencia

Enterovibrio

Shigella sonnei

Triple Sugar Iron Agar Media (TSI)

[glucose : lactose : sucrose]
disaccharide

1 : 10 : 10

aerobic

↑
Slant

butt.

↓
anaerobic

Slant → oxidative deamination

↓
peptides (AA) broken down to NH_3

↓
 $\uparrow \text{pH}$ [Red]

Butt \rightarrow fermentation $\rightarrow \downarrow \text{pH}$ [yellow]. 130

Lactose \oplus



Initially, glucose fermentation occurs
 \downarrow
yellow colour.
 \downarrow

Later glucose is finished.

$$\text{Lac} \oplus = \text{Acid}$$
$$\text{Acid}$$

Then Lac \oplus organism comes

Lactose metabolism



So $\text{pH} \downarrow$



Again Yellow

Lac \ominus



Initially glucose fermentation occurs
 \downarrow
yellow colour



Later glucose finished



Then Lac \ominus can't metabolise Lactose



No new acid production

So, oxidative deamination continues. \therefore out any neutralisation.



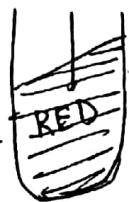
$\uparrow \text{pH}$



Red colour

Non-Fermenter

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Non-fermenter = $\frac{\text{Alkaline}}{\text{Alkaline}}$

Oxidative Decarboxylation occurs.

Vibrio → Lac⁻ glucose Y fermentation (+)



for Vibrio - TSI + McConkey Req.

as in TSI Selective Lac⁺ organisms

H₂S producing Enterobacteriaceae

TSI media since contains Fe

Fe + H₂S → Black colour. ⇒ BLACK COLONY

Salmonella

Proteus

Citrobacter

Edwardsiella

Media used

- (1) Bismuth sulfite citrate sulfide
- (2) Deoxycholate citrate agar (DCA)
- (3) Xylose Lysine deoxycholate (XLD)
- (4) Salmonella shigella Agar (ss)
- (5) Hektoen enteric agar (

Fe

- (6) Lysine agar
- (7) TSI, Kligler Fe Iron (KIA)

- (8) SIM (Sulfide Indole Motility medium)
- (9) Lead Acetate Agar

tube

KIA contains - glucose; Lactose

1:10

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It is used in Vibrio non-endemic area as it doesn't contain sucrose as sucrose is for Vibrio.

E. COLI

UTI - fimbriae P, F, S, Type 1.

Lower serotypes O₁, O₂, O₄, O₆, O₇

→ Screening -

M/E

Griess Nitrate Test

Conf' culture of urine.

Significant ~~bacteremia with~~ bacteriuria

$\geq 10^5 \text{ cfu/mL}$

Midstream urine

Suprapubic aspiration.

→ KASS CONCEPT

except → Gram +ve Org. } $10^2 \text{ to } 10^5$
→ Catheterised patient }

Urine culture

std. loop technique

Miles & Mishler

Quantitative

1 mL of urine in media

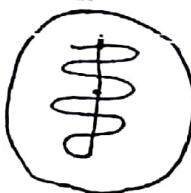
Semi-Quantitative

4 mm internal diameter

0.001 mL approx.

Coulter Counter..

Colony counter.



Media -

1) Mc Conkey + Blood Agar

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↳ used so that Steph aware if present

2) CLED (cysteine Lactose electrolyte Deficient media)

• differential media

• prevents swarming of bacteria

• promotes growth of Steph. & Candida

(enriched media not required)

Diarrhoea

Serotype

Virulence Factor

1) STEC — Lambda like Stx1 or Stx2 encoding

EHEC

bacteriophage

2) ETEC

CFA, LT + ST (plasmid)

3) EPEC

EPEC adherence factor (plasmid)

Locus for enterocyte effacement (LEE) -

chromosomal pathogenicity island

4) EIEC

Invasion, Intracellular spread.

cell to cell transmission (plasmid)

5) EAEC

Adherence + toxin genes (chromosomal)
plasmid)

EPEC

- Paediatrics
H/C infant children

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Non-inflammatory Diarrhoea

& Adhesion on HEK293/HEP cell lines



Enteroadhesive E.Coli

EFT

Δ - Serotyping

ETEC

- T- Traveller's Diarrhoea H/C

CFA

Toxins

LT (Labile)

↑ cAMP

Δ - Rabbit ileal loop
ligation test



Ballooning

BIKEN test

(ppt)

ST (Stable)

↑ cGMP

Infant or mice ileal
loop ligation

Ag detected by ELISA
using mouse Ig

* Serotyping for ETEC

ETEC causes non-inflammatory diarrhoea

EIEC → atypical E.Coli
Non-motile / Lac-

135

Inflammatory Diarrhoea

Δ - Sereny Test [conjunctivitis in Rabbit Eye]
Serotyping

EHEC → Inflammatory Diarrhoea & Blood.
due to stx (shiga like toxin) or verocytotoxin

↓
haemorrhagic colitis

HUS → EHEC H/C

90% association.

$Stx_2 > Stx_1$

H/C cause of outbreak.

Δ - For Screening ⇒ Sorbitol MacConkey media

↓
EHEC doesn't ferment Sorbitol

Serotyping

EAEC → BRICK STACK PATTERN

Non-inflammatory Persistent Diarrhoea

KLEBSIELLA

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Necrotising Pneumonia & Empyema

Lower Lobe → Lobar pneumonia

HAI. (hosp.-acquired)

Hypervirulent Klebsiella pneumoniae (hvKP)

1) Hypermucoviscous phenotype

2) ↑ dissemination

3) String Test (>5mm long) → also seen in Vibrio

4) Klebsiella - lysine decarboxylase

	E.Coli	Klebsiella
Motility	(+)	(-)
Urease	(-)	(+)
Mucoid colony	(-)	(+)
IMViC	++--	--++

Indole

Indole Test

~~Indole Tryptophan~~

↓ Tryptophase

Indole ; Pyruvate acid - NH₃

↓ red complex aldehyde group

in KOVAC or Schillie reagent

(+) → E.Coli

(-) → Klebsiella

Enterobacter

Hafnia

Serratia

Methyl Red

pH indicator = Red at 4.4

(+) for E.Coli

Voges Proskauer

Acetoin detected.

(+) for Klebsiella
Enterobacter
Hafnia
Serratia

Citrate

Utilisation of citrate

when citrate utilised, Green → Blue colour change

SALMONELLA

Salmonella enterica subspecies enterica

Serotypes

Typhoidal

S.Typhi M/c

S.Paratyphi A

S.Paratyphi B

Enteric fever, Neutropenia

fecal route Non Typhoidal (NTS)

S.Typhimurium M/c

Gastroenteritis.
Neutrophilia.

Serotype - mottle except S. Gallinarum +
S. Pullorum

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NTS

Salmonella → Toxin → causes endothelial intestinal epithelial damage

↓

IL8

↓

Neutrophilia

No blood or mucus

Pus (+) in stool.

Rx - not required

no antibiotics

Since antibiotics will lead to ↑ fecal shedding

↓

'outbreak'

ENTERIC FEVER

Salmonella multiplies in Peyer's patches [MALT]

↓

Spleen, Lymph node,

Bone marrow.

↳ it gets blocked

leading to neutropenia

Neutropenia

Hepatosplenomegaly

Lymphadenopathy

∅

other features
confirmation

Rose spots

Bradycardia

Step ladder fever.

Pea soup stool [consipation/diarrhoea]

For conformation

Blood Culture - 1st + 2nd week 1:10 dilution. 139

Widal test - 3rd wk onwards.

WIDAL TEST

Tube agglutination.

To detect O + H Ab in pt serum using Widal Ag.

T_O - 'O' Ag of S. Typhi

T_H - 'H' Ag of S. Typhi

A_H - 'H' Ag of S. paratyphi A

B_H - 'H' Ag of S. paratyphi B.

'O' Ab appears early 'H' Ab persists longer

'O' agglutinin is granular → round bottle **FELIX TUBE**

'H' . . . fluffy → conical **DREYER TUBE**

Mirror is used for looking at the agglutination.
at the bottom.

16 tubes are used. (4x4)

Widal Ag extraction - (O-901 strain of salmonella
used)

'O' Ag → Heat stable

Smooth strain (LPS=OAg)

grow in phenol Agar. H-O variation. [loss of flagella]

ethanol / chloroform

Not total loss

H Ag → Heat labile
 Rough strain (No LPS - No Ag) 140
 S → R variation (loss of virulence)
 "old culture"
 autoagglutinable.
 grow in Craigie tube.
 H antigen and merozoite
 salmonella

 → formalin is used for killing
 Semisolid agar media

Factors affecting Widal

1) Endemicity (single serum sample)

cut off titre

'O' Ab $\geq 1:320$

(or) 'H' Ab $\geq 1:640$

4 fold rise in titre

2) Anamnestic Reaction

Transient rise in widal titre due to some other infection in apparently a previously infected pt.

Ig G is responsible for it

3) False +ve Reaction

due to some other infec'

4) Rx

5) Vaccination. — TAB (killed), Ty 21a (Live)] polyvalent

Vi - polysaccharide - mono valent

↓ Doesn't affect widal titer.

from 2 yrs to any age group.

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Rx Ciprofloxacin - DOC
2nd line - ceftriaxone

CARRIER

By: Bile culture

Stool culture

Vi Ab detection using viAg (source is Citrobacter)

Serotyping in salmonella

Kauffmann White Scheme.

Folie negatively white

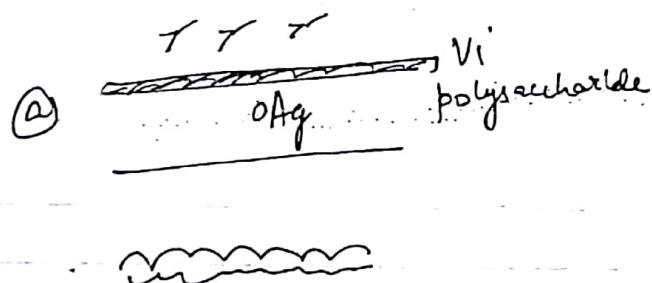
Serotyping is removed by

V → W variation achieved

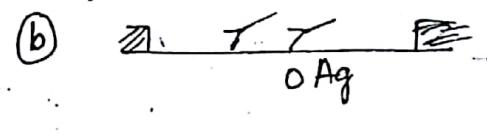
by heating

Best Specimen - Bone Marrow culture Non-O agglutinable

(painful but ↑
sensitivity)



[V forms] fresh isolates.



[W forms]

O agglutination.

SHIGELLA

142

- Non-Motile
- Produces Gas (due to pga formic acid)
- No Gas :- can't cleave formic acid (analogous)
- Infective Dose - 10-100 bacilli
- Causes outbreak

S. DISENTERIAE

chromosomally encoded toxins

↓
verocytotoxin (ctx)

enterotoxin

neurotoxin (acts on blood vessels)

12 serotypes

3.

S. FLEXNERI

6 serotypes → X + Y Variant

More common in India

S. BOYDI

18 serotypes

S. SONNEI

no serotypes → antigenically homogeneous

[Most common] worldwide

✓ Colicin Typing (Bacteriocen Typing)

Invasion → By Virulence marker Ag (plasmid)

143

A - Stool culture. (Salmonella + shigella)

* Enrichment media [liquid = inhibition].

↳ Yerme thionate Broth

Selenite F Broth.

SS Broth

* Selective media → media for H₂S detection

PROTEUS

Swarming

Pleomorphic

produce urease → cause UTI & calculi
— (struvite)

A - PPA test (Confirmation for Proteus, Providencia & Morganella)

↓
Phenyl pyruvic acid

Diene phenomenon for typing

↓
using swarming

(Test) (Control)

If they overlap.
↓
same strain

If they are separated by a
line
↓
different stain

YERSINIA PESTIS

144

Toxin → No virulence (O)

VF :-

VW antigen

F₁ protein (plasmid)

Coagulase

Phospholipase

Virulence +.

helps bacilli to survive at
27°C in flea midgut

Plague (Black Death)



- Bubo
- pneumonic bubo → if enters lung
- Septicaemic
- Outbreak

Endemic Area

① Beed - Latur

② Kohlba

③ Rohra (Shimla)

④ Uttarakhand.

A - Wayson Stain

Safety pin Bacilli

due to bipolar staining



Ghee Broth - Stallactite Growth.
at 27°C

YERSINIOSIS

Gastroenteritis & merentary involvement

145

Y. enterocolitica M/c

Y. pseudotuberculosis M/c mimics appendicitis.

M/c Bacteria & mimics appendicitis \Rightarrow Pasteurella multocida

A of yersinosis -

Stool culture on buffer saline. Q.
(cold enrichment)

Selective Media \rightarrow CIN (Cefsulodin Irgasan Novobiocin media)

CALYMMATOBACTER (KLEBSIELLA) GRANULOMATOSIS
Intracellular

- Donovanosis / Pseudobubo / Pseudo elephantiasis.

[no lymphadenopathy]

- Safety pin appearing Bacilli in mononuclear cell (PUND cells) \rightarrow Diagnostic

H. DUCREYI

Pain.

Chancroid \rightarrow Painful

Requires only Factor X for growth.

A ~~& School of fish~~ or Tramtrack or Rail Road

Extracellular

Gram -ve Coccobacilli

146

BURKHOLDARIA PSEUDO MALLEI

shows safety Pin Appearance

Gram -ve.

Safety Pin Appearance

Vibrio parahaemolyticus

Pathovar

Burkholderia Pseudomallei

Calymmatobacter

Yersinia

FERMENTORS

VIBRIO

VIBRIO CHOLERA

Comma shaped & single Polar Flagella.

Virulence

→ Endotoxin - no virulence

→ Exotoxin

CT (cholera-toxin) Molt Imp

ACE (Accessory Cholera exotoxin)

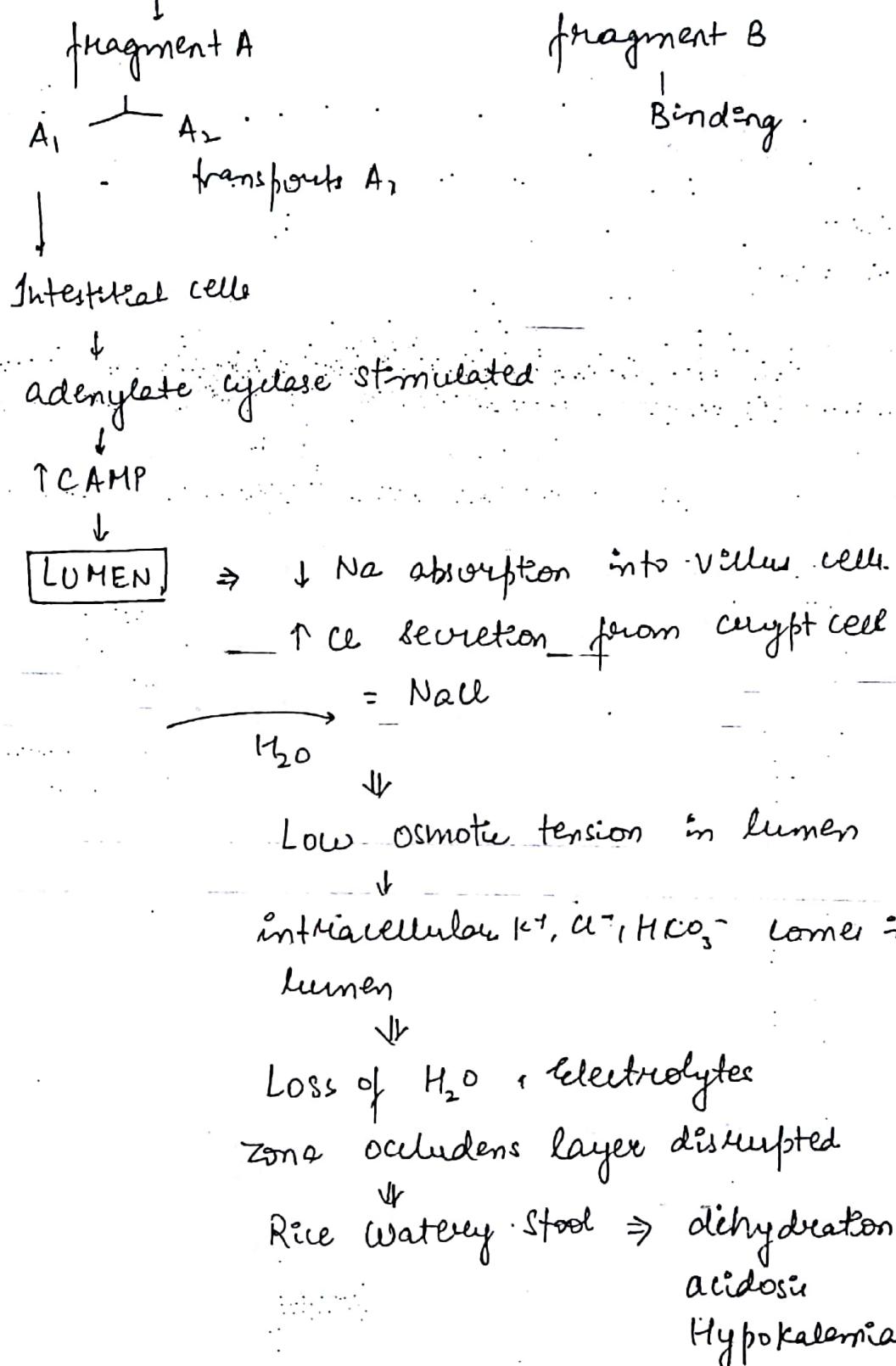
ZOT (Zona occludens toxin)

TCP (Toxin coregulated pilus)

found in O₁, O₁₃₉

Cholera Toxin

147



Diagnosis

- ① Stool Culture
- ② Enrichment Media → Alkaline Peptone Water
Monsur Taurine-cholate broth

① Transport Media - Cary Blair
VR media
autoclaved sea water

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② Selective Media - Bile Salt Agar
GTTA (Gelatine Tetrachloroethylene Tellurite)

TCBS (Thiosulfate Citrate Bile Salt
SUCROSE)

V. cholera → yellow colonies

Halophilic vibrio → green colonies [Halophilic].

↳ V. parahaemolyticus → causes necrotizing enterocolitis

Kanagawa phenomenon ← [β hemolysis on High salt Agar
containing Blood Agar Media
[Wagatsuma Media]]

2) V. vulnificans.

Darting Motility.
other tests -

- String Test
- Oxidase +
- Cholera Red Reac +
↳ Nitroso indole formed.

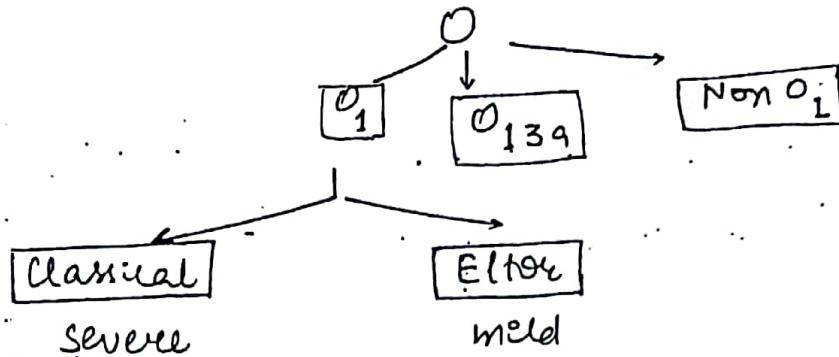
* Epidemic Period → Source is Human

* Interepidemic Period → Source is Crustaceans in sea water

Typing of Vibrio

149

Serogroups



1st & 6th pandemic

originated from Bengal

7th pandemic

from Indonesia

Best for typing

Biotyping

(VP Test)

→ +

⊖ — +

⊖ +

③ R

IV II

Mukherjee'

Bioserotype Biovar - classical El Tor

— Classical

Ogawa | Inaba Hikazima

AB

Ac

ABC

El Tor

O I H.

O139

- capsulated

↳ capsule contains O₁₃₉ Ag



• 1st isolated in Chennai.

- Bengal Strain → 1992-94 outbreak in Bengal

It caused outbreak.

So, Non-pandemic Strain. 150

O₁₃₉ is antigenically, epidemiologically distinct from O₁

Clinically similar

No cross immunity seen.

Vaccine

Killed vaccine oral vaccine is classical. Eltor & Ogawa + Inaba

↓
↑ Mucosal Immunity

'Copro Ab' Q.
Ab

in stool.

NON-FERMENTORS

PSEUDOMONAS

[Obligate Aerobe]

→ environmental

Colonies have shiny "Gun Metal" appearance.

fruity odour / Grape odour

NLF, Oxidase +ve

Grows at 42°C

Pigments :-

Pyocyanin - blue.

Pyoverdin - green

Pyorubin - red

Pyomelanin - black

Virulence Factors

① Pili - Adhesion to cell 151

② Flagella - Adhesion, motility, inflammation

③ Capsule - Biofilm

④ LPS - Bind to CFTR for internalisation
Antiphagocytosis
Inflammation

⑤ Type III Secretion System - ExoS
ExoU
ExoT
ExoY
Antiphagocytosis

⑥ Type II Secretion system - ExoA - inhibition of EF2
Cytotoxicity of phagocyte

⑦ Phospholipase - Cytotoxicity of Phagocytes

⑧ Proteases - Proteolytic activity

Complement System Evasion by Pseudomonas

Elastase & alkaline phosphatase

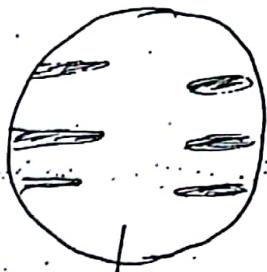
↓
degrade C1q C3b/C3b

△ - Cetrimide Agar - Selective Media

Typing - Bacteriocin Typing [Image]

Difference antimicrobial Agents secreted¹⁵² by different strains

Shigelle Sonnei - colicin
Klebselle - Klebocin



Grow strain in between
1 remove it next day

Chromofom application.

Now put again strain.

Growth differ due to
antimicrobial substance released.

Rx

» Neutropenic host or MDR

↓
Cefipime

② Non-neutropenic host

↓
Monotherapy

↓
Ceftazidime

↓
Combination

↓
Pip/taz or
CARBEPENEM &
Amikacin

BURKHOLDERIA PSEUDOMALLEI

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(1) Capsulated

(2) Most virulent amongst Non-fermenters.

If has all ~~sepsis~~ secretion system + capsule.

(3) ~~Melioidosis~~ → Pulmonary Infe' Abcès

pneumonia

necrotizing lung Disease

skin ulceration

Lymphadenopathy

(4) Bioterrorism - Cat II agent

(5) Long Latent Period → 'Vietnam Time Bomb Disease'

(6) Δ → Gram -ve

Safety Pin

Culture - Ashdown media -

cornflower head wrinkled

Purple colonies

DOC - Meropenam / TMP-SMX.

STRENUSTRÖPHOMAS MALTOPHILIA

(Non-Fermenter)

154

Gram-ve.

Motile (polar)

Environmental

HAI

A - culture → Lavender Green

✓ Gray colour colonies on blood agar

Oxidase (−)

Oxidation of glucose + maltose

Doc- TMP-SMX * Ticarcillin - clavulanic acid

ACINETOBACTER

Environmental, HAI

Non-motile

Oxidase (−)

R_X - same as pseudomonas.

Non-Fermenter (ZOONOTIC)

BURKHOLDARIA MALLEI

Equine (Horse) → Glanders

BURKHOLDARIA CEPACIA

Causes Cepacia Syndrome

Associated in sepsis

A - Colistin containing media agar.

Pneumonia in Cystic Fibrosis & mucoid colonies

→ *B. cepacia*

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BACTERIAL VAGINOSIS

Caused by *Gardnerella*

Bacteroides

Mobiluncus sp.

↓ *Lactobacillus*

Nugent Score $> 7 \Rightarrow$ Diagnose

Based on less no. of *Lactobacillus* & more pathogens.

Clue cell - epithelial cell studded = Gram -ve bacteria

Whiff test - Amine (fishy odour)

pH > 4.4 .

CHROMOBACTERIUM VIOLECIUM

Purple coloured.

Lead to lung & wound "infection"

Gram -ve

SERRATIA Marcescens

Pseudomonas

CAPNOCYTOPHAGA

156

Slow growing

Coprophilic

Gram-ve

Fusiform / filamentous

Gliding Motility

Modifies chemo tactic ~~factor~~ activity of neutrophile

Fulminant sepsis in aplenia or albinism.



fusiform
Gram-ve

HACEK

Haemophilus parainfluenzae (Mc) s/o isolated from.

HACEK endocarditis

A. actinomycetemcomitans - Prosthetic valve
endocarditis.

Cardiobacterium hominis → aortic valve

Eikenella corrodens → Least common cause of HACEK.
endocarditis.

Human Bite → clenched fist injuries Q.

Klebsiella kingae - septic arthritis in children. (M/)

Purpure rash similar to meningococcus

LEGIONELLA PNEUMOPHILA

BCYE

157

- * Charcoal in the ~~BYE~~ media used to isolate Legionella pneumophila
 - ↳ (Buffered charcoal Yeast Extract)

↓

detoxifying agent

[Black colour]

- * Contaminated water (amphibian algae).

↳ aerosol through A/c aspiration.

→ No person to person → transmission



A - ① Neutropenia

② Culture on BCYE media [charcoal for detoxification]

↓

Monoxygene media

(requires 1 biological agent in media)

③ used in fracture culture

④ Immunofluorescence - sensitive method

H. INFLUENZAE

158

Pfeiffer's Bacillus

aerobe

oxidase +

pleomorphic

seep sample - coccobacilli

CSF - filamentous.

1st organism - entire genome sequenced

2 months to 3 years - No Ab to PRP

Biotype I is M/c cause of meningitis

Virulence

capsule → 6 serotypes (a to f)

Type b → capsule has PRP

[95% infection] (polysaccharide ribitol PO₄)



causes invasion.

5% of infection - Non-capsulated or
Non-Typable strains

Vaccine - Hib

(monovalent polysaccharide conjugate
vaccine)

A. Require X , V factor for growth.

① Culture

159

- a) chocolate agar - factor V released
- b) Blood agar \supseteq *S. aureus*
- c) Nutrient agar \supseteq disc of X , V factor
- d) Levinthal agar (capulated strains produce
iridescence)
- e) Fildes Agar - peptic digest. of blood on nutrient
agar.
(Best)

— Satellitism in *H. influenzae* is due to factor V .

~~satellite~~ Bigger colony around *S. aureus*.

↓
It stimulates producⁿ of V
factor

H. AEGYPTIUS

Koch's weeks Bacilli or Brazilian purple fever
Egyptian conjunctivitis Red eye.

BORDETELLA PERTUSSIS (Gram-negative coccobacilli)

Whooping cough → inspiratory stridor
100 day fever

No animal reservoir unlike B. Bronchiseptica

Violence -

- ① capsule → No Role in Virulence
- ② Pertussis Toxin (Type I, IV) - ~~secretory~~ system activates adenylate cyclase → ↑ cAMP
- ③ Tracheal cytotoxin
- ④ Adenylate cyclase Toxin
- ⑤ Diphtheria toxin
- ⑥ Endotoxin
- ⑦ Adhesins (Type V) → FHA, pertactin, fimbrial secretory system BckA protein

۱۷

↑ Histamine

↑ insulin.

Lymphocytosis. → huge

1. + Thrumb Print appearance

② Regan Lowe → Mercury Drops on Biseected Pearl

Charcoal + 10% horse blood +

Cephalexin) Regan Lowe \Rightarrow Transport media

③ Bordet-Gengou → Potato Blood Agar
 ④ Ab detect by ELISA 161
Vaccine
 whole celled → PCR 15461 - PT promoter
 acellular region gene
contains ↓ more safe
 PT = pectin ~~toxin~~
 FHA1 - fimbriae
 FHA2 } filamentous
 FHA3 } haemagglutinin Ag
 Pectactin.

Thiomersal - preservative

✓ FRANSICELLA

TULARENSIS

Zoonotic

- Transmission → ticks/ deer fly or direct contact → ~~turkeys~~/musk rats penetration of skin M/c inhalation, ingestion

Culture media

✓ chocolate agar

✓ MTM (mod. Mayer Martel)

✓ BCYE

Gram -ve Cocco bacilli

A - Serology:

M/c

Rx - Resistance to β lactamase

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streptomycin / gentamicin

tetracycline

chloramphenicol ..

FQs

CAT A agent of Bioterrorism.

(BSL III req for culture).

M.TB

- ① Obligate aerobe
- ② Micro-aerophilic → M. Bovis.
- ③ Virulence factor
 - CORD factor.

$\Delta \rightarrow$ M/E → ↓ sensitivity

10^4 bacilli/ mL of sputum is req

a) Zn Stein - slender, long, curved.
[for confirmation] beaded

b) Auramine Rhodamine - Direct fluorescence ably

↓
Bind to mycolic acid

Highly sensitive → used for screening

2) Culture

LJ media

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Egg containing media = alpargine
Malachite Green (Selective)

M.Tb → Eugonie growth

M.Bov → Dysgonie growth (sparses)

Petroff Method → sputum processing

(15-20 min)

9% NaOH
N-Acetyl Cysteine (mucolytic agent)

↓
centrifugation at ↑ RPM

↓
BSL (II) (Biosafety Level)

Bect CB NAAT (Cartridge Based Nucleic Acid Amplification Test)

- PCR
- BSL - (II)

INNO LiPA (Line probe assay)

Reverse Hybridisation technique.

20 DNA probes of different Mycobacterial species are immobilized on nitrocellulose strip. The amplicons (amplified DNA) are hybridized & to the probes

Chromogenic Reagents is used instead of radioactivity

GIMD (Genotype Mycobacterium Benefit)

NASBA (~~NA~~ where RNA is amplified ¹⁶⁴ reverse hybridized = RNA probe
Nucleic acid sequence based assay
Only 5 species detected

IS6110 - Best for Genotyping

Best Culture Technique - BACTEC NGIT

- ↓
- ✓ fluorescent
- ✓ 2 days - 7 days

Latent TB

Mantoux Test

→ 5 TU of PPD-S

or 1 TU of RT₂₃ strain ← RNTCP

after 72 hrs ↓

in L forearm.

>10mm induration along short axis
on L forearm

↓

Positive → exposed

Quantiferon Gold Test

IFN-γ Release Assay

IGRA

Whole blood

→ Lymphocyte → T + IFN-γ

stimulate w/ M-Tb Ag e.g. -

ECAT 6
CFP 10

+

exposed

M-Tb T, F

⇒ less false +ve

Q No. cross Rx E BCG or atypical Mycobacteria
or NTM or MOTZ
except M. Kansasi.

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ATYPICAL MYCOBACTERIUM

↳ Runyan classification

Photochromogens → Pigments in Light
M. Kansasi

M. marinum [swimming pool / Fish tank granuloma]

Scotochromogens → Pigments in dark
M. scrofulaceum
M. szulgai

[coughing in dark]

Non. Photochromogen - No pigments.

M. ulcerans → Buerger Ulcer

MAc → < 50 CD₄ cells in HIV

M. avium

M. intercellulare (Batey Bacilli)

Rapid growers - M. chelonei } cause post-trauma
(< 7 days) M. fortuitum } abscess

[loan + fortune = rapid growers]

MTB

Atypical

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① Niacin	(+)	(-)
② Amyl Amyl Sulphatase	(-)	(+)

M. LEPRAE [acid fast staining in 5% H₂SO₄]

SSS (short, stout, straight bacilli)

[Image]

acid fast B. in pallisade

> 50 bacilli - globi

in histiocytes - FOAM cells

Specimen - SSS - split skin smear (6 specimen → 4 skin, 1 ear lobe, 1 nasal mucosa)
+ nt in edge of lesion
Ab to PGIL1

M/E - ↓ sensitivity 10^4 bacilli / gm of tissue.
IOC

Culture - Bert Armadillo (natural infection)

M/c - Mice (suckling)



Ghymectomy

(to induce lepromatous leprosy)

~~LEPTOSPIRA~~ Leptospire → ?

Dark ground Microscopy → Treponema

Light microscopy → Borrelia

SPIROCHAETES

Spiral organisms & endoflagella → cork screw motility¹⁶⁷

T. Pallidum → venereal Syphilis.

1^o stage → Hunterian chancre

↓
painless, indurated

self-limiting. c'm. 4-6 weeks.

2^o → Dissemination.

after 2 to 6 months.



Condyloma

Latent → No sign + symptom

3^o → complications.

Aortic aneurysm

Tabes Dorsalis

Δ -

1^o stage → ① Dark Ground Microscopy

↑ only reflected light through from object
through spiral condenser

Resolution power not increased.

only optical illuminator used.

② EIA (Enzyme Immuno Assay)

IgC

2^o stage → TPPA (Treponema Pallidum Particle Agglutination)
(Early) gelatin
- Most sensitive & specific Overall
Used for confirmation.

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Screening → VDRL
slide flocculation Test



Cardiolipin Ag [Calcutta Ag]
(derived from ox heart)

Reactive → clump.

[Image]

Non-reactive → scattered

Using microscope → floccule seen.

RPR

Cardiolipin + carbon particles
Particle agglutination based test

Nichols Strain → Pathogenic Treponems

TPI not safe but ↑ specificity

TPHA - haemagglutination assay
↓ sensitivity

FTA-ABS Reiters strain → non-pathogenic treponems

(Difficult)

+
→ Abnormal if Non-specific Abs in serum
then ↓

Nichols strain used

[Indirect immunofluorescence]

LEPTOSPIRA

169

① Curved at 1 End. ? interrogans.

② H/c. Zootoxo disease.

③ JR → Rats. Rainfall, ricefield

Rat urine → penetration of skin.

affect Liver & Kidneys

Jaundice haemorrhage fever & myalgia

WEIL'S DS / Andaman Fever

A → Culture on Korthoff, Fletcher, EMJH media

② Ag detect in urine

③ Typing → Serum is used.

(Leptospira difficult to grow)

Reference Lab

Macroscopic agglutination Test (MAC)

↳ Killed Leptospira Ag used to detect Serogroups

↓
Serotypes having similar Ag

Passive Agglutination

Serogroups detected.

Microscopic agglutination test → Serotype detected

Live Leptospira

immobilization test → Dark Ground Microscope used

BORRELIA

170

Thin → can be seen in Light microscope

Causes Relapsing Fever



B. Burgdorferi → LYME's Disease

Erythema chronicum Migrans

[Image].

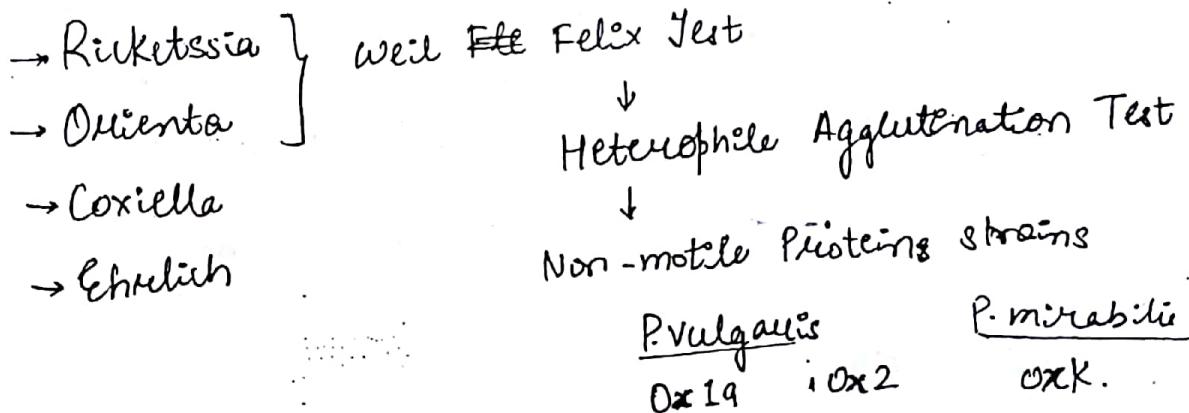
↓
Concentric centrifugal Rash.

P66 Ab by western Blot Assay

(Non-Specific But Best)

25/2/18

RICKETTSIACEAE FAMILY



Russia is endemic for Typhus Fever

TYPHUS FEVER

Epidemic

Louse

causative

R. Prowazekii

Endemic

Flea

causative

R. Typhi

Brill-Zinsser¹⁷¹

(Recurrent)

P. Prowazekii

"Some of the elderly not suffering from Disease"

S. Spotted Fever

Tick Borne.

Ox. - Rocky Mountain - R. Rickettsiae
Fever

Indian tick typhus }
 Japanese " " } R. conori
 Siberian " " }

mite → Rickettsial pox → R. Akari ♀

Scrub Typhus - Ixombiculid → oriental

Oxk

mite (Larva)

tsutsugamushi

Epidemic Typhus

R. Prowazekii

Neil Moore or

Tunica vaginalis

test

Endemic Typhus

⊕ in R. Typhi

C✓
A✓
T✓

Q FEVER / ABBATOIR / QUEENSLAND FEVER

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⇒ Tick act as reservoir → transmit to sheep, goat, cattle but not to humans

→ transmission to humans by respiratory route - aerosol from soil, tissue or during parturition. only rickettsial infection can be transmitted from anthropod i.e. man to man → resp. route

→ Acute Infection → Influenza-like

→ Chronic " → Infective Endocarditis M/c

→ Risk Factors - Pre existing valve anomaly
- Immunocompromised

Δ -

→ Culture only in cell lines [BSL 3 lab]

- A Immunofluorescence 10c
Giemsa stain.

PCR

≤ Coxiella survives Holdere's Method by parturisation:

↓
63°C for 30 min.

[Flash method - 72°C for 13-15 sec].

Ehrlichia → Motula (vacuole in phagocytose)

Sennetsu → raw fish.

Chaffensis } tick → affects monocytes

Phagocytophilia } → affects granulocytosis.

CHLAMYDIA

Gram -ve Cell Wall But no' peptidoglycan. (no neuraminic acid)

Lack ATP synthesis \Rightarrow Energy Parasites

Infectious form \downarrow Elementary Body
(50 - 330 nm)

Δ form \rightarrow Reticulate body

Pathogenicity :-

C. Trachomatis \rightarrow ~~H.B.~~ H.P. Body (Hechtader Prowazek)

serotype A-C \rightarrow Trachoma
D-K \rightarrow Inclusion Conjunctivitis
NGU

\rightarrow Fitz Hugh Curtis
 \rightarrow Reitter's syndrome
conjunctivitis \downarrow Polyarthritides
urethritis

LGV \downarrow \rightarrow Lymphogranuloma Venereum
 \downarrow painless ulcers painful bubo

Δ - TRIE SERROVARS.

M/E \rightarrow Giemsa, Giminez, Zasteneda,
Machiarella. LGV \rightarrow Miyagawa corpuscle

\rightarrow culture on Macay cell lines

\rightarrow Ab detection by ELISA

\rightarrow NAAT by PCR.

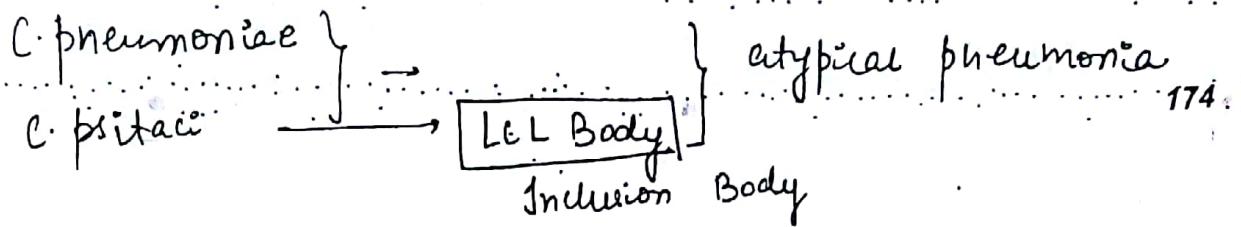
LGV Serovars. \rightarrow Fluorescent Test

Ab detect' by Micro Immunofluorescence
assay

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① Inclusion Body
(Reticulate Body)

② Macay cell Lines



Typing :-

- 1) LPS → Genus Specific Ag (CF Test)
- 2) Envelope Ag → Species specific Ag
- 3) Outer Membrane protein → serovar or serotype specific Ag [Micro IF test]

MYCOPLASMA

PPLD or Eaton Agent
[pleuro-peritoneal like org]

My fried egg glided in dining while eating

M/c cause of atypical pneumonia or walking pneumonia

✓ No cell wall
✓ Sterols +nt in cell membrane

No turbidity in liquid media → also seen in B. anthracis.

Δ

① Culture in media + cholesterol

↳ Diene stain of isolates [Methylene Blue + Azure]
fried egg colony

② Serology → Heterophile agglutination Test

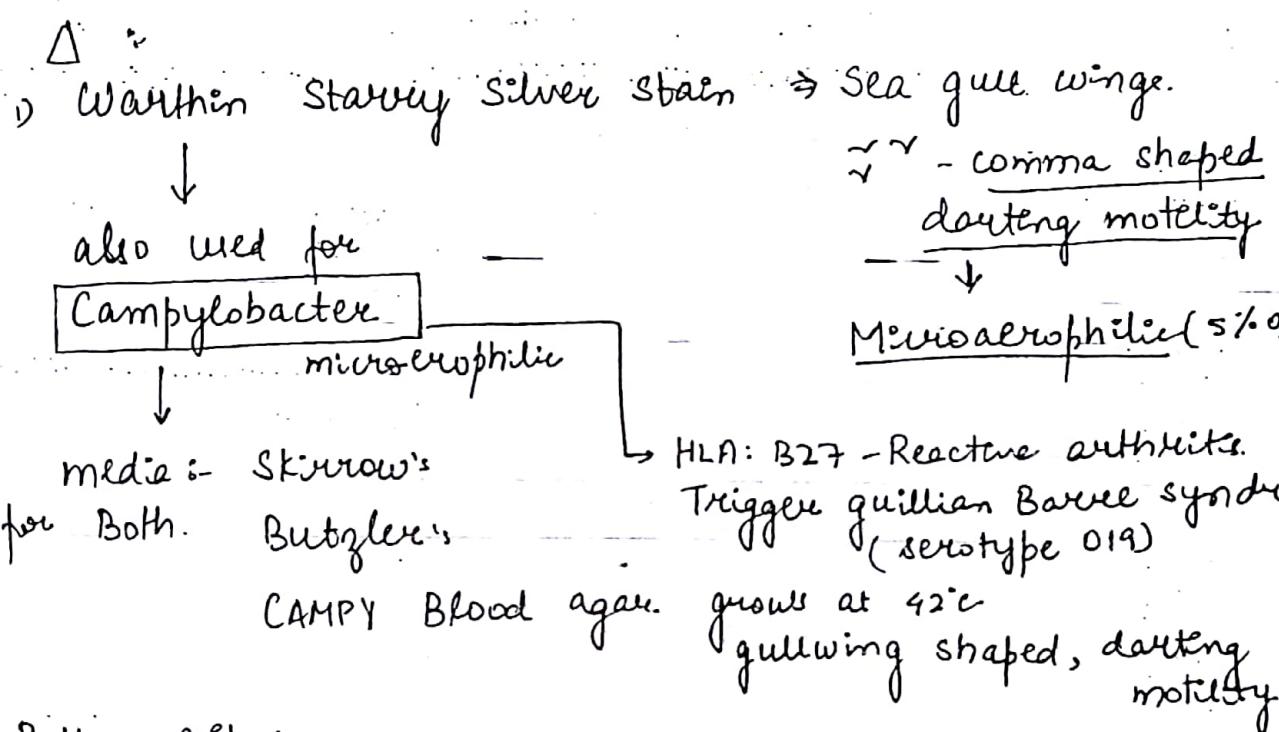
a) Cold agglutination → O-ve RBC at 4°C.

b) Streptococcal MG Test

HELICOBACTER

- 1) Deep in mucous layer on epithelial side (pH - 7.4) 175
- 2) Proteases → modify gastric mucosa - reduce diffusion of acid.
- 3) Urease activity → Ammonia - buffering of acid
- 4) Complement system evasion by H. Pylori

Protectin inhibits MAC → C₅-C₉



Pathogenicity :-

Helicobacter → peptic ulcers

↓

Adenoma / Maltoma

Campylobacter → Gastroenteritis + blood.

↓
Reservoir (poultry)

Gold std. for A of Helicobacter → Urea Breath Test

Biopsy + urease → for dysplasia

BARTONELLA BACILLIFORMIS

176

CARRION's DISEASE

Oroya Fever - Acute phase

fever + other constitutional symptoms

Severe anaemia, jaundice, Hepatomegaly

Lymphadenopathy, myalgia

← [Female Lutzomyia
(sandfly)]

Verruga Peruana - after resolution of Oroya fever

[Image] Non tender,

red to purple

modular lesion.

Δ :-

M/E → Warthin Starry or Giemsa stain

↙ Bacillus in RBC

Culture on blood agar at 30°c

Ab detect

PCR.

B. QUINTANA →

French fever (5 day fever)

By louse.

cause outbreak

B. HENSELAE → Cat Scratch Disease → Cervical LN

Basillary Angiomatosis

Cat Bite → Pasteurella

RAT BITE FEVER

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Spirillum Minor

3-5 mm

Helical spiral org.

Rat bite fever (Sodoku)

Local lesion regional gland
swelling. clear water.

Culture - inoculation in guinea
pig / mice

Flagella at Both ends

Streptobacillus moniliformis

Gram(-ve)

Aerobic

Pleomorphic bacteria that
forms irregular chain of bacilli
interspersed = finger
enlargements

L-forms seen.

Rat bite fever - Septic fever

Blotchy

Petechial rash
purple Polyarthritic

Ingestion of milk -
epidemics of

Haverhill
fever

Culture - Trypticase soy
enriched = 2% blood.

BRUCELLA

Zoonotic

(from animal milk)

affects the reticulo-endothelial System

Intracellular

Triad

- 1) Undulating fever
fever = night sweat
- 2) Arthralgia
- 3) Hepatosplenomegaly

Hepatosplenomegaly

Lymphadenopathy

Malta or undulating fever (typhoid like illness)

- Neutropenia

DOC - Doxycycline + streptomycin.

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A - ⁸⁰ Casteneda Blood culture.

'syphilitic media'

Serology

→ coombs test

→ Std. agglutination test

11 tube. dilution.

"PROZONE" Phenomenon.

PCR.

→ Gold Std

Silver Impregnation → Levanitis / Fontana
for spirochaetes.

PARASITOLOGY

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Protozoa
(unicellular)

Helminths
(multicellular)

Platyhelminth
(flat)

Nemathelminth.
Round.

Cystoder
(segmented)

Trematodes

Nematodes

(unsegmented)

Incomplete
alimentary canal

complete
alimentary
canal

Digestive, excretory
reproductive spaces
are separate

[No cyclops]

"NO alimentary canal"

Hermaphrodites except
schistosoma

Separate sexes

INTESTINAL PROTOZOA

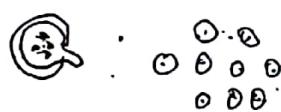
ENTAMOEBA HISTOLYTICA (pathogen)

[Image]

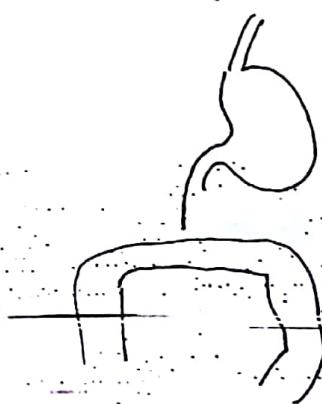
Infective form \Rightarrow Quadrinucleate Cyst

Excitation occurs in ileum (\uparrow pH)

cyst \rightarrow Trophozoite.



MIC site - Sigmoid colon
Caecum (M/c)



Virulence factors:-

- ① Galactosamine Lactin → binding
- ② Protease → degrade collagen
- ③ Calmodulin & phosphatase A → inhibit phagocytosis
- ④ Thioredoxin Reductase → degrades toxic O₂ N[•] radicals.

Q: Distal colon → encystation occurs.

Trophozoites → cyst

Diagnosis:-

- ① M/E of stool. (wet or iodine or trichrome stain)

	<u>Pathogens</u>	<u>Non-Pathogen</u>
Trophozoite	15-20 μm active RBC or cellular debris in trophozoite [erythrophagia] Nucleus central Ectoplasm & Endoplasm - [thin] [granular]	20-30 μm sluggish Bacteria → trophozoite eccentric
Cyst	6-15 μm 1-4 nuclei Uninucleate stage	15-20 μm 1-8 nuclei Binucleate stage No difference Q.
Glycogen Mass		
Chromatoid Body		

Δ Rectal Biopsy \Rightarrow Flank shaped ulcer.



Other ways of A

- ① Nested PCR
- ② zymodeme assay \rightarrow isoenzyme pattern on electrophoresis
- ③ galactosamine Lectin Ag detection by ELISA] IOC for Gal/Gal NAC ELISA [invasive amoebiasis

EXTRAINTESTINAL AMOEBIASIS

~~Hem~~ Hematogenous Route

Liver (H/c) Lung Brain

— \hookrightarrow Posterosuperior quadrant of R lobe

Cutaneous (directly)

\hookrightarrow cutaneous amoebiasis.

① OM/E of Liver aspirate \rightarrow Anchovy sauce pus trophozoite \downarrow



Trophozoite at periphery

② PCR \rightarrow most sensitive

③ Radiology

④ Clinical diagnosis

FREE LIVING AMOEBA

Found in natural water source

Acanthamoeba \rightarrow 1° lung infection

haematogenous

Granulomatous Amoebic encephalitis

Keratitis (contact lens)

182

Δ - CSF → Both Cyst + Trophozoites

Naegleria → flagellated amoeba

travels via olfactory n/v

pierces cribriform plate

1° amoebic meningoencephalitis

↓
fatal

Δ → CSF only trophozoites

- A of free living Amoeba

→ culture on non nutrient agar = E.coli → Trots.

→ M/E of CSF

• H.E stain - [Cyst + trophozoite → acanthamoeba
Trophozoite → Naegleria.

• Immunofluorescence [Image]

GIARDIA INTESTINALIS

Infective form - Quadrinucleate cyst

Excystation takes place at the Jejunum - M/c site

Trophozoites bind in ventral sucking disc

↓
disrupt brush border enzymes

↓

Malabsorption

Steatorrhoea

foul smelling stool
Anaemia

Megaloblastic
Pernicious

(↓ IF)

Diarrhoea

Stool → "Loose Greasy" yellow colour

183

Δ -

- ① Stool M/E for trophozoite cyst H/c
wet mount, trichrome, Kohn, Giemsa
- ② Direct Immunofluorescence assay → aztreonam
- ③ Ag Detection by ELISA, rapid immunochromatographic assay
- ④ Other test - Entero test → strong ingestion + then
Duodenal Biopsy...
NAAT

Each Trophozoite has 4 pair of flagella [Image]

- [1 ant]
- [1 middle]
- [2 post]

BALANTIDIUM COLI

Ciliated.

LARGEST PROTOZOA

Infects Pig, humans (occasionally)

Asymptomatic (M/c)

Persistent Intermittent Diarrhoea.

Doc - Tetracycline

Δ - stool M/E → trophozoite (cyt rarely seen)

Tissue staining in H&E after endoscopy

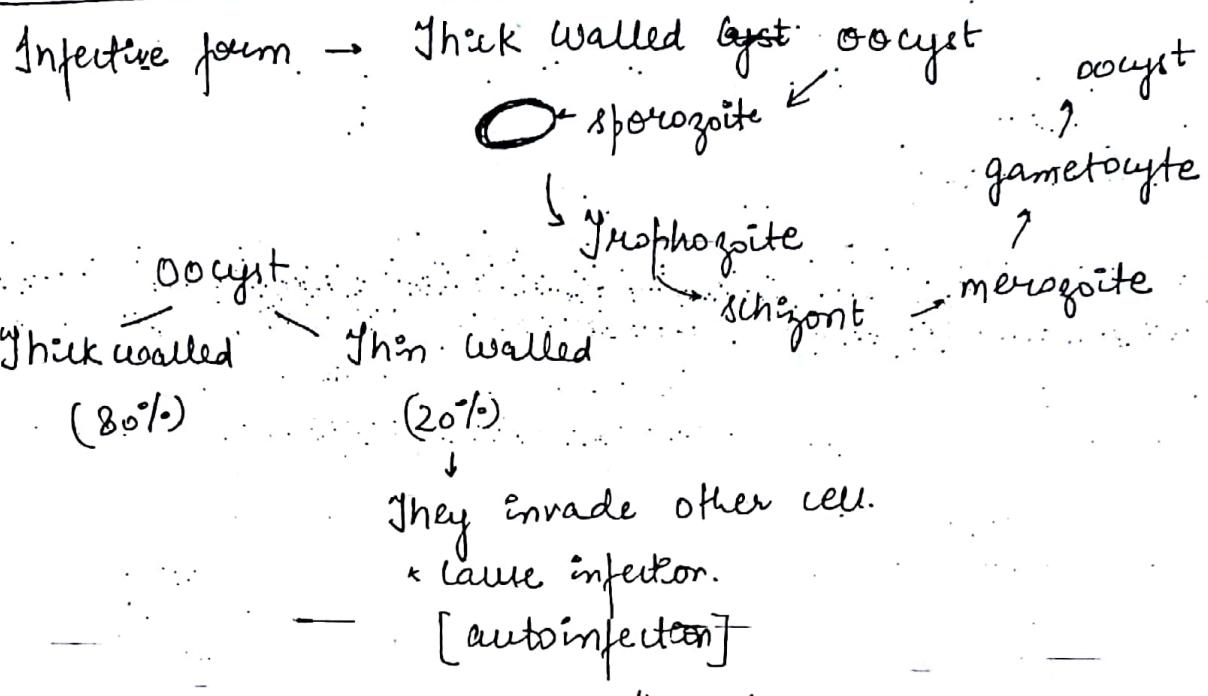
[Differential Interference Contrast → for cilia]

COCCIDIAN INTESTINAL PROTOZOA

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Diarrhoea in HIV pt + extremes of age

CRYPTOSPORIDIUM PARVUM ($6\mu\text{m}$)



No t/t, only improve immune system Persistent Diarrhoea

CYCLOSPORA ($10\mu\text{m}$)

autofluorescence seen.

ISOSPORA

Δ :- ① ~~Kinyoun~~ stain [Modified ZN stain] → Acid fast cold technique oocyst

[cryptospor - $6\mu\text{m}$]
 [cyclospora - $10\mu\text{m}$]
 [Isospora - $25-40\mu\text{m}$]

A Decolorizing Agent → 0.5% H_2SO_4 .

* ghost cell → unstained cells

② Immunofluorescence Assay by auramine

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MICROSPORIDIUM

↳ earlier considered coccidian

- Fungi
- cause diarrhoea + ocular infec'
- Spores seen.
- Δ :- "Weber Stain"

PLASMODIUM

Pigments

- ?

Peripheral Blood Smear - Gold Std

Δ :-

RBC

Enlarged + Pale

Schuffner's - yellow

Single infection

~~1 RBC~~

1 RBC = one parasite

All stages

P. falciparum

Normal

Maurer's dot - Black

Multiple infec'

1 RBC - multiple parasite

Early + Late stages



SEQUSTRATION

Q ↓

Vascular endothelial cells

Mech. of

a) cytoadherence.

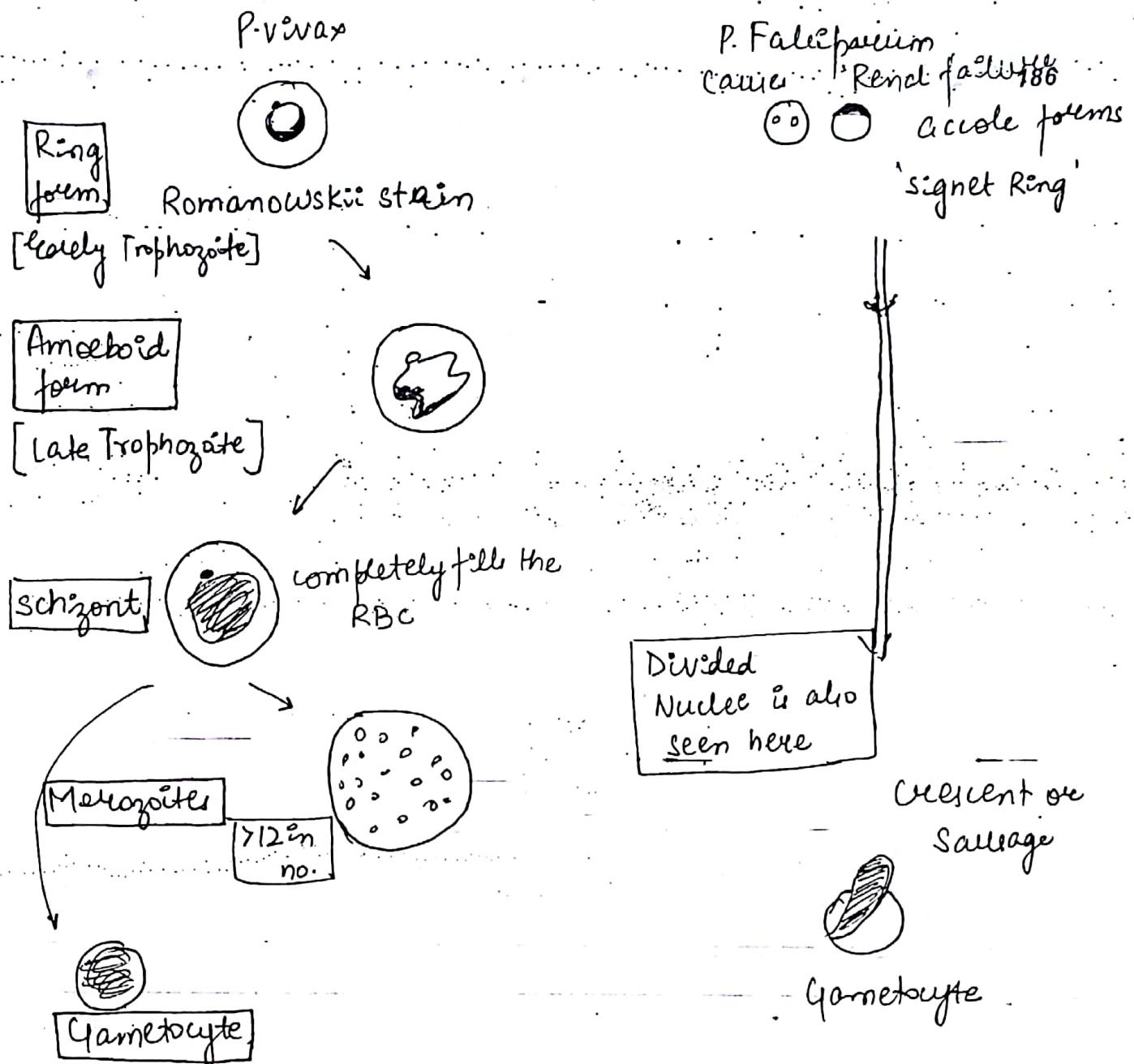
- adhesion molecule

b) Rosette formation

- 2-3 parasite stick together

c) Agglutination - surface adhesion on RBC





P. MALARIAE → cause **Nephrotic Syndrome**

RBC - normal

Band forms



Basket forms



Merozoites

≤ 8 No.

Zeimann's Dots
(light Brown)

P. OVALE

Similar to vivax

James Dot's

Dark Brown

187

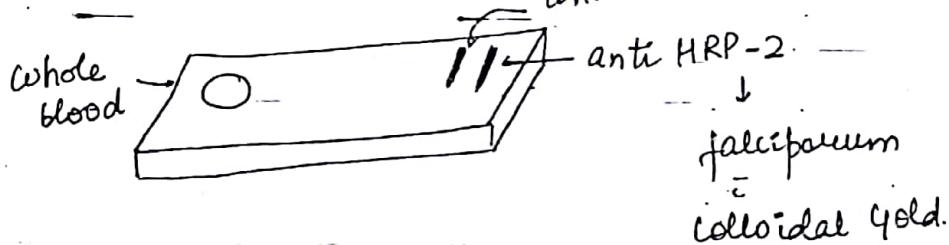
Quotidian → Shenton Mulligan

Thick Smear → Screening

Thin Smear → Species Identification

Other tests for Diagnosis

1) Rapid Immuno chromatographic array
anti PLDH (all species)



2) Quantitative Buffy Coat Technique

Acridine Orange for fluorescent

Relative except falciparum + malariace

LIFE CYCLE

Infective form → sporozoite

sporozoites
↑ Salivary gland

ookinete

↑
oocyst

zygote

MOSQUITO

gameteocytes

↓
liver cells

Hypnozoites

exo erythrocytic cycle

Merozoites

RBCs

Pre-
erythrocytic

trophozoite

Schizont

* Transfusion Malaria / Mother to child.

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M/c ↓
 Trophozoite
 Schizonts
 Merozoite

short I.P. as No pre-erythrocytic cycle

* Screening of blood

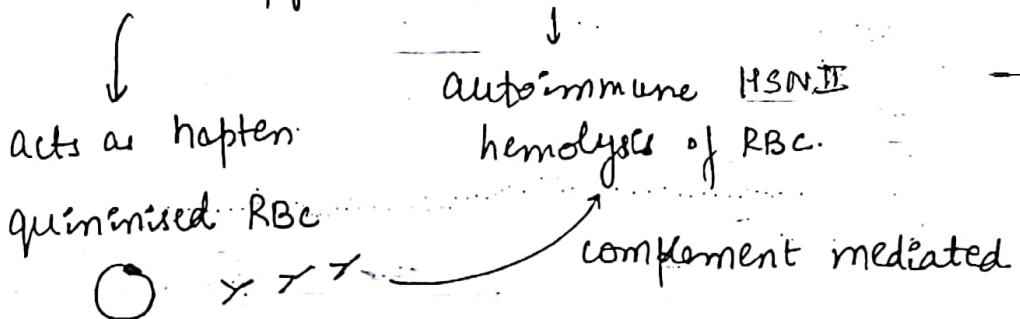
Serology → Rapid. Immuno chromatographic Assay

Advantage of Rapid.

No expertise required.

Falciparum

quinine therapy. - Black Water Fever



quinine → causes hypoglycemia
 hence should be accompanied by 25% D.

BABESIA MICROTI - Protozoa invading
 RBC

Tick borne

R/F → Splenectomy

Maltese cross tetrads → seen in RBC.
 A form

HAEMOFLAGELLATES

Leishmania
Trypanosoma

LEISHMANIA

L. donovani
L. infantum
L. chagasi - new world.

→ Visceral Leishmaniasis.

Infective form → Promastigote (flagellated form)

deposited on the skin by

Sand fly

Phlebotomus

- Old world

Lutzomyia

- new world

Penetrate the skin
through trachea

AMASTIGOTE

Spleen
95%

BM

65-80%

LN

50%

↑↑ IL-10

↓

inhibits TH₁

↑↑ TH₂ response

↓

IL-6 released

Proliferation

Massive splenomegaly
Kalaazar.

① M/E of Bone Marrow.

Bert
Test

Macrophage → LD Body

190

1st kinetoplast on amastigotes

↓
Dot & Dash

[Image]

② Napier Aldehyde / antimony test.

Serology

③ Montenegro Test

Negative except in Sudan

④ Culture on NNN medium.

↓
Rosette formation of promastigotes.



Cutaneous LEISHMANIASIS

L. Tropica M/c

Delhi Boil or Oriental Sore

Mucocutaneous - L. Braziliensis M/c

L. Mexicana complex

PKDL - Nodular Lesion on face after Visceral Leishmaniasis

↓
East Africa [Sudan] - after 2-6 months

Indian Subcontinent - (Bangladesh) > 3 years.

India - W.B., Bihar, Assam

TRY PANOSOMA

T. CRUZI

T. BRUZZI

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T. CRUZI

causes Chagas Disease



Chagoma

Infective form → Trypanomastigote

deposited by

Triatomae / **Reduviid** Kissing or
Assassin.



Chagoma - skin lesion.

cardiomyopathy

megalolon

Romana Sign ⇒ U/L Periorbital oedema

Δ - Amastigote in (heart) tissue.

C-shaped Trypanomastigote in blood

T. BRUZZI

causes Sleeping Sickness

Infective form → Trypanomastigote

vector - **Tsetse Fly**



inhibition of Insomnia Receptors.

Winter Bottom Sign ⇒ cervical L.N. enlarged

Δ - NO amastigotes.

ELONGATED PROMASTIGOTES

192

glycoprotein switching
↓

immune evasion.

TRICHOMONAS

VAGINALIS

[Image]



1 morphological form →

4-5 ant. flagelle + 1 along- undulating body

greenish frothy D/c

Whiff Test tve

LMP TOXOPLASMA

A ♀ = H/o recurrent abortions present to ANC in her

1st trimester TORCH screen

↳ IgM

IgG

+

Toxo +

-

Rubella -

-

CMV -

+

HSV -

+

Next Management ?

a) Start spirogyccn.

b) advise MTP

c) IgG avidity test

d) IgA detection

Toxoplasma Gondii

Transmission through cats & canines.

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Infective form \Rightarrow oocyst in cat faeces

↓
ingested

Man - accidental host:

↓
Bradyzoite

↓
Tachyzoite (motile)

↓
tissue cyst (dormant)
(Bradyzoites)

Sign, symptoms develop in immunocompromised,

♀

HIV pt.

CD₄ < 50

Reactivation.

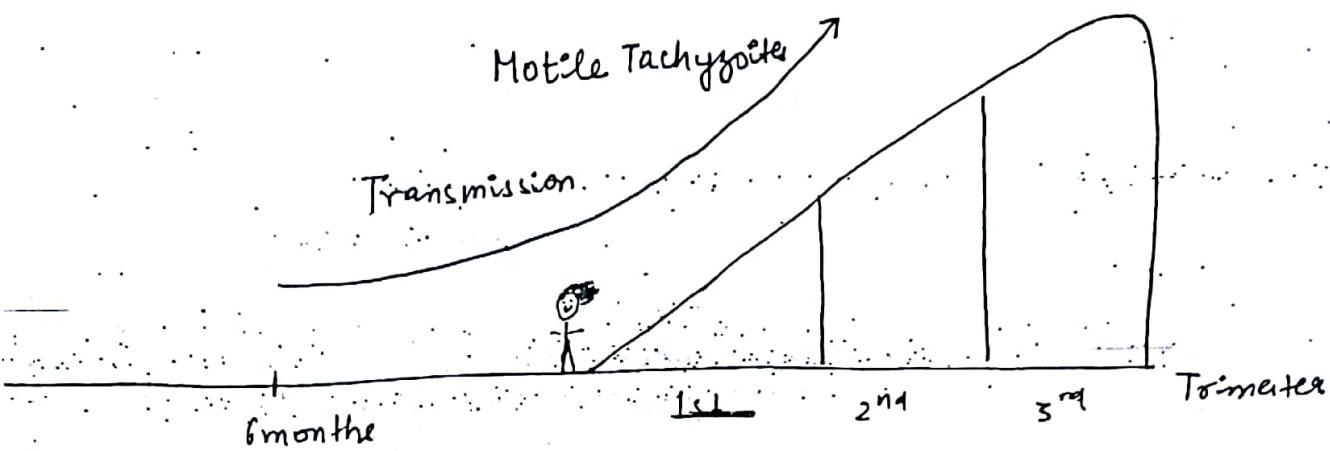
Fetal encephalitis

- ① No IgM, low IgG titre
- ② MRI - Crescent shaped multiple Ring enhancing lesion.

Eccentric target sign

③ PCR of CSF:

↓ spiramycin - Dog

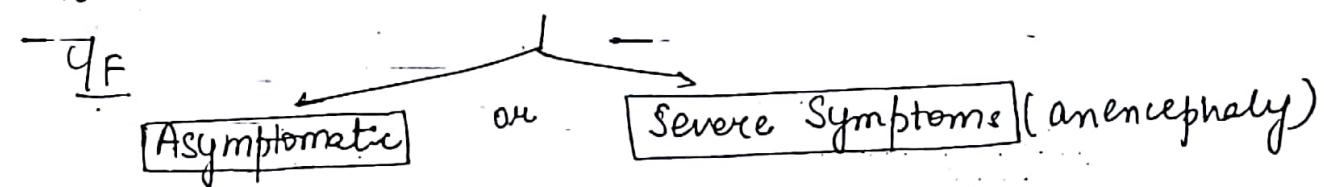


Beyond 6-months

no tachyzoites

↓
no transmission

← foetal affection more in
earlier part



Late sequelae of
chorioretinitis

↓
Lead to Blindness

Risk Assessment In Cong. Toxoplasmosis :-

By Serology

Ig G +.

Ig M -

↓

Past Infect
[Not significant]

high

Ig G +

Ig M +
↓
(stays for
 ≥ 1 yr)

Ig G avidity Test
(measures ~~affinity~~) affinity)

Low

Recent
infection

Ig G - Ig G -

Ig M + Ig M -

↓

Susceptible
↓
Pneumocystis

↓
Preventive

IgA detection

Low IgG avidity

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↓
more sensitive test for recent inf.

Sabin Fieldman dye Test.

to detect Ab

↓
Not recent Inf.

CYSTODES

Developmental Stages

Egg - Coracidium - Procercoid Larva - Pleurocercoid Larva

Trematodes Developmental Stage

Egg - Miracidium - sporocyst - Madiae → Cercar. - Metacercariae.

Definitive Host

T. Saginata
Diphyllobothrium
Hymenolepis
Dipylidium.

Intermediate Host

Echinococcus
Sparganum
Coenurus

Both Definitive & Intermediate - T. solium.

Paratenic Host

- no development of parasite in this host

1) Prawn → Angiostrongylus cantonensis

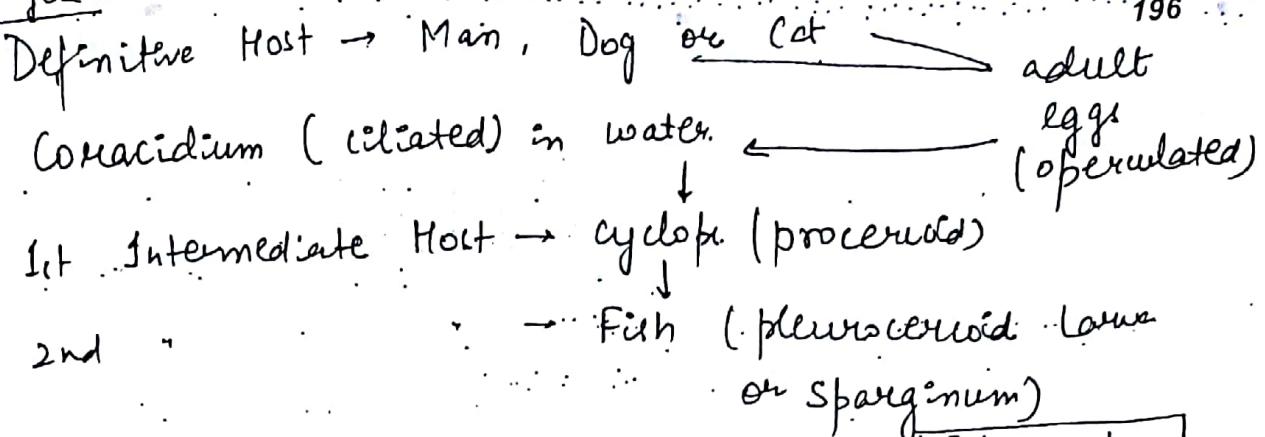
2) Big Fish → pleurocercoid larva of D. latum

3) Fish → Gnathostoma spinigerum

4) Man → pleurocercoid larva of sparganum Q

DIPHYLLOBOTRIUM LATUM

Life Cycle



Pathogenicity:

Asymptomatic Infecⁿ - M/c

Abdominal Pain

Pernicious Anaemia (absorption of B_{12}).

LARGEST CYSTODE

A - Eggs 45-700 (upto 100 μm)

[Brown] = [Operulum] at one end.
[Knob] at another

Eggs of *Diphyllobothrium*, *Fasciola* + *Fasciolopsis*
 $> 100 \mu\text{m}$, operculated, indistinguishable.

SPARGANUM

Definitive host → Dog, Cat → adult

Coracidium in water



1st intermediate host - cyclops (plerocercoid larva)
→ infective form ← M/c

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2nd intermediate host → fish, Reptiles, Amphibians [plerocercoid larva]
infective form

Man - plerocercoid larva (parasitic form).

TAENIA

INTESTINAL TAENIASIS

Infective form - cysticercus (single scolex)

T. Solium

(Pork)

2.5 m

T. Saginata

(Beef)

10m.

Asymptomatic
Malabsorption

Δ - scolex → hooks 13 No. in rostellum ⇒ T. Solium

Proglottide

Eggs

Bile stained

Stilettos



NEUROCYSTICERCOSIS

Infective form - eggs (contaminated vegetables)
of T. Solium

↓
oncosphere m/s - M/c

cysticercosis

← Brain - epilepsy.

Absolute Criteria

↓
Cysticercus in tissue.

" by fundoscopy

" by Radiology (Mc)



If cysticercus not seen, then Other Criteria :-

as other radiological signs

b> Ab detection : by ELISA

c> Clinical evidence

d> Epidemiological Indicators

COENURUS (T. multiceps or T. serialis)

• Multiple scolices

• Coenuri of T. multiceps → found in eye & Brain

• T. serialis → in Subcutaneous tissue.

ECHINOCOCCUS (HYDATID CYST)

Ectocyst (Outer cuticular layer)

acellular

Laminated hyaline membrane

appears as white of hard boiled egg

Endocyst (Inner germinal layer)

cellular

Vital layer of cyst

Gives rise to benign capsule & sclerite

Secretes the specific hydatid fluid - forms outer layer

E. URANULOSUS → Hydatid cyst

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E. MULTILOCULARIS - Alveolar cyst
(cyst Metastasis)

E. VOGELI - Poly cystic Ds

Dog Tapeworm [Definitive Host]

A - 1) Casoni Test (anaphylaxis)

2) Ab detection by ELISA

3) CT scan IOC

Screening for Echinococcus

- for E. multilocularis

DIPYLIDIUM

Infective form :- flea harboring cysticercoid [Dog, Cat, Man]



Solid cyst = scolex

M/c in children

Asymptomatic M/c

HYMENOLEPIS

Infective form - Egg → cysticercoid → Adults.

One HOST (No intermediate host) ♀

A - Eggs - 30-40 μm

Non-Bile Stained

6 spicules = knobs = HEXACANTH

Similar to
egg of
Taenia

TREMATODS

Q. The infective stage of trematode causing swimmer's itch is Cercaria.

Eggs as Infective Form
200
Hymenolepis
Echinococcus
T. multiceps
serialis
T. solium (Ncc)

SCHISTOSOMA

Separate Sexes

Male - gynaecophoric canal

~~ope~~ Non-operculated

No Rediae

Inf. form \rightarrow Cercaria

Life Cycle

OTHERS

Haemaphilidite

operculated

Rediae

Metacercaria



Ingestion

penetrates skin

Metacercaria

Ingestion

Man (adult)

Eggs (water)

Miracidium
(motile)

Snail

Sporocyst

Rediae

fish

Clonorchis
opisthorchis

crab
paragonimus

water
plants

fasciola
fasciolopsis

metacercariae
(encystment)

All Trematodes are Oviparous

Hepatomegaly → egg granuloma

Katayama syndrome → oviposition, a serum sickness like illness.

Fibrosis (Symmer's)

due to Th1 response

Swimmer's itch [cercarial dermatitis]

in *S. mansoni*, *S. japonicum*.

S. Haematobium

Egg - 100-150 μm , non-operculated

S. Haematobium - terminal spine

Ca Bladder.

S. mansoni - Lateral spine

S. japonicum - Spine inconspicuous

CLONORCHIS OPISTHORCIS

Only egg of Trematode $< 100 \mu\text{m}$ = 15-30 mm
Operculated in shoulder.

Cholangiocarcinoma

~~PARAGONIMES~~

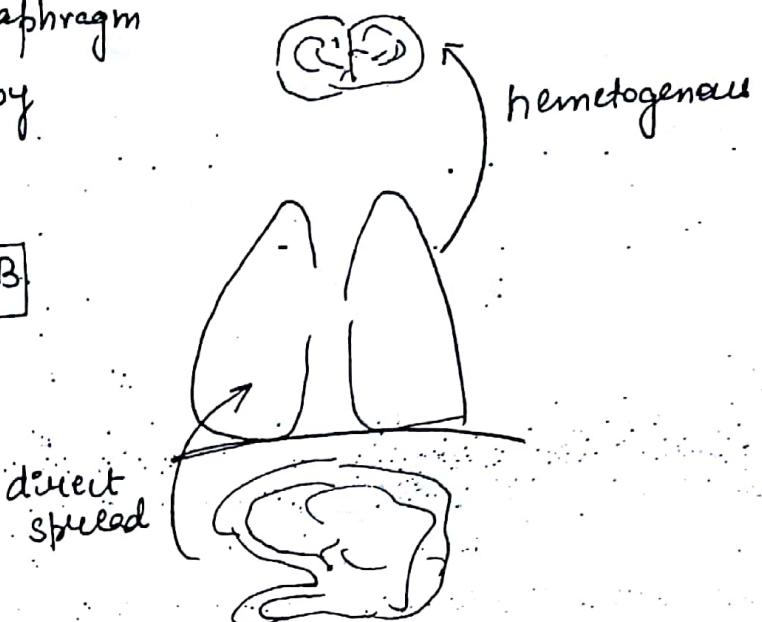
PARAGONIMUS

202

It directly pierces Diaphragm

But spread to brain by
haematogenous spread

Paragonimus Minus P.TB

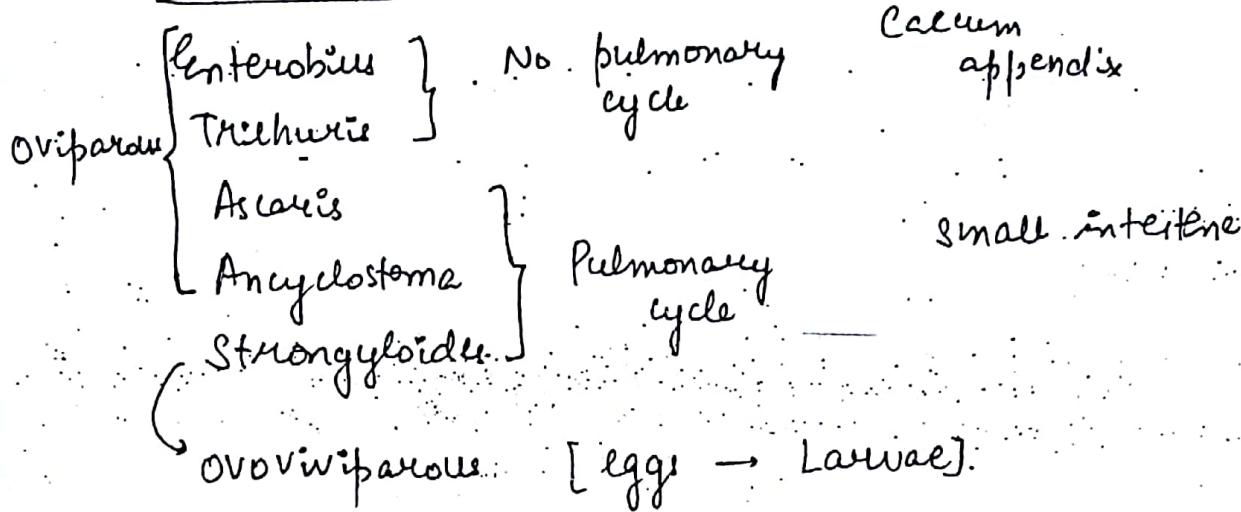


A- Golden Brown Eggs in the Sputum.

NEMATODES

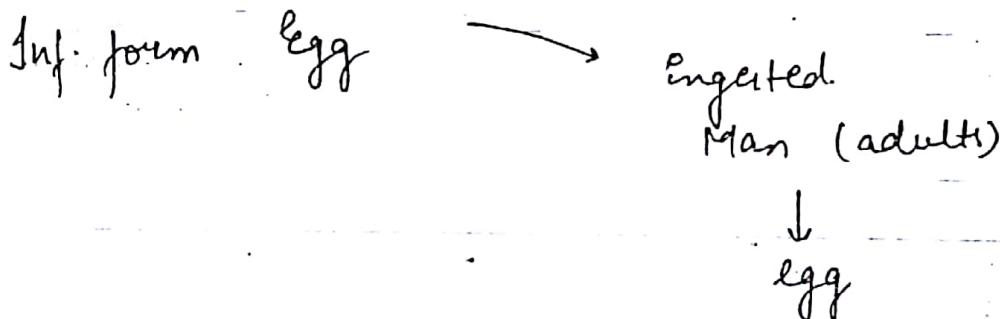
203

INTESTINALES



Larvae in Stool → *Strongyloides*

ENTEROBIUS — (Pin worm) —



Adults - Pointed / sharp end → **Perritus.** M/c symptom

Δ Scotch tape (NIH swab)
 ↓
 Perianal region.

Egg :- 70 µm
 Non Bile stained
 Plano-convex

TRICHURIS (Whip worm)

204

Asymptomatic Infec'

Anaemia

Rectal Prolapse.

A - Eggs in Stool.

70-80 μm

Bile stained

Barrel shaped

Bipolar plugs.

ASCARIS

Infective Form - Egg = Rhabditiform larvae



Larvae

(penetrates into circulation)



Heart



Lungs

Epiglottis



(swallowed)

small intestine
(adults)

Adults - Fecundity ↑ (No. of eggs laid/worm/day)
(2.4 lakhs)

Cause intestinal obstruction.

Larvae → Loeffler's syndrome

A - adults (male are shorter & have curved ends)

Egg 50-60 μm

Bile stained

Rugosity



Non-Human Ascaris Worms

Toxocara canis H/c

205

" catie



Visceral Larva migrans.

ANKYLOSTOMA

Infective form - Filariform Larvae

↓
penetration of skin

Adults - 0.2 mL of blood/worm/day

Anaemia

Larva - Loeffler's syndrome

A. Egg - 50-60 μm

Non Bile Stained

4 blastomeres



Non-Human Ankylostoma

A. Braziliensi → Cut. Larva Migrans

H/c "creeping eruptions"

STRONGYLOIDES

Inf. form - filariform Larvae

↓
penetration of skin

Parthenogenetic female - lay eggs & out males
fertilized

~~Indirect development in soil~~ → ♂ : ♀

Dermatiti LARVA CURRENS - larva migrate @ 10cm/hour

A ① Bernmann Funnel technique

206

② culture by Harde - More filter paper technique / agar plates.

Filariform Larvae → Sharp (B) side



Rhabditiform larvae - Blunt 1. end

TISSUE NEMATODES

Filarial worms Trichinella spiralis

FILARIAL WORMS

Inf. form → 3rd stage Larva
↓ mosquito

Tissue

Signs + symptoms → due to adult
except ONCHOCERCA.

A - Microfilariae in blood.

except onchocerca.

BRUGIA MALAYI

Fragmented Nucleus in tail end of microfilariae

Nucleus - Blue

Cytoplasm - Pink



W. BANCROFTI

Nuclear material do not extend to the tip.

LOA - LOA

Nuclear material extends upto loa loa

MANZONELLA

Unsheathed.

Nuclear material upto tip

207

ONCHOCERCA

Simulium (deer fly)

↓
Inf. form Larva

↓
adults in tissue nodules Q
over bony prominences

also causes \hookrightarrow RIVER BLINDNESS

A. adults in tissue

culture of larvae from skin snips

ANGIOSTRONGYLUS CANTONENSIS (rat lungworm)

leptomeningitis

Infective form - Ingestion of 3rd stage in molluscs.

venereal larva migrans in Brain - H/c causes the

Angiostrongylus COSTARICENSESIS

Abdominal angiostrongyliasis H/c

Symptoms mimic appendicitis.

ANISAKIASIS

Anisakis simplex \rightarrow ~~Pseud~~ *terranova decipiens*

3rd Stage Larva in fish.

Surgical Resection.

Gnathostoma spinigerum

208

3rd stage Larva in fish or poultry

Eosinophilic meningoencephalitis

Migratory cutaneous swellings of the eye & visceral organs.

Surgical Resection.

TRICHOSTRONGYLUS

Infection - Ingestion of Larva (vegetable)

Ingest far less blood than hookworms

Asymptomatic. (M/C)

Heavy infections - anaemia + eosinophilia

Stool exam

Eggs resemble hookworm eggs but are larger.
(85 by 115 μm)

TRICHENELLA SPIRALIS

Infected form - Encysted Larva in yak or polar bear

↓
adults in intestinal mucosa

↓
migration + encysted larvae
cause signs + symptoms

Δ - Eosinophils

↑ CPK

Ab detection

M/s Biopsy (at the tendon insertion)

Lemon Sign (nurse cells)

Bachman Intradermal Test

209

M/s involved → EOM, Bilepr Jaw, Diaphragm.

Larval Load - < 10 larvae/gm of tissue

↓
asymptomatic

> 50

→ fatal.

Egg Load → Chandler's Index

7300 → Major Public Health problem
seen in Hookworm

AUTOINFECTION

C - Cryptosporidium, Capillaria philippinensis

H - H Nana

E - Enterobius

S - Strongyloides

T - Tenia Solium

MYCOLOGY

210

Cell Wall - Chitin

Mannan

Glycans

Cell Membrane - Ergosterol

β -glucan assay - all fungi except
Cryptococcus.

SDA

isolates \rightarrow Lactophenol
cotton Blue.

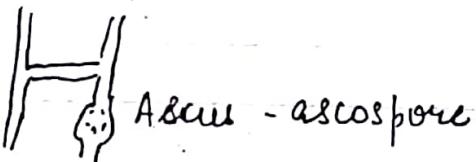
Calcofluor white \rightarrow fluorescent

Classification (sexual reproduction)

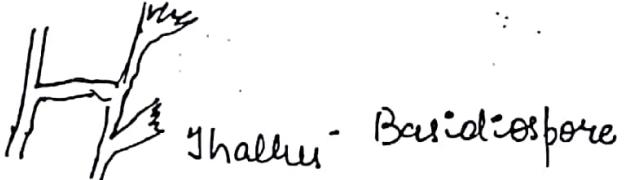
Zygomycete



Ascomycete



Basidiomycete



Deuteromycete No sexual spore

'Fungi Imperfici'

Morphology

Yeast - cryptococcus

Yeast like - Canida

Dimorphism - $\begin{cases} 37^\circ\text{C} & \text{Yeast} \\ 25^\circ\text{C} & \text{Mould} \end{cases}$

PUJ *Sporothrix schenckii* - Himachal [Rose Gardener's D]

Penicillium marneffei - Manipur.

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Histoplasma → Eastern }

Blastomycosis }

Coccidioidomycosis → Western }

Paracoccidiomycosis → South American

Moulds

Rest

OPPORTUNISTIC FUNGAL INFECTIONS

M/c - *Candida*

CANDIDA

Endogenous —

↓
CMI ↓ → mucocutaneous

Neutrophil ↓ → invasion

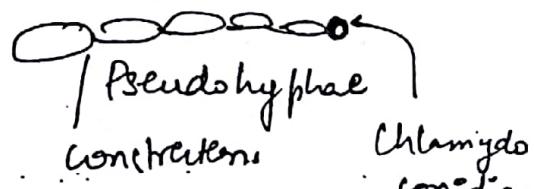
↓
True & Pseudo hyphae

True Hyphae → apical elongation when candida is
[Raynaud Braun grown in serum for 2 hours
phenomenon]

Pseudohyphae → failure of daughter buds to separate

↓
Seen in corn meal Agar Germ Tube → HYPHAE

[Nutritionally Deficient media]



Candida
Albicans

Germ Tube +

Chlamydospore +
Chlamydioconidia

Candida albicans

8-%

C. Dubliniensis - Non-albicans & can produce
germ tube, chlamydiospore ²¹²⁰

NON- ALBICANS

C. Tropicalis

~~(A)~~ Nodal blastospores

spores from buds

C. parapsilosis



(B) Nodal + internodal

C. Glabrate
(mucoid)

No pseudohyphae

C. Krusei



cross matchstick

CRYPTOCOCCUS

It causes infec' in HIV pt.

Virulence factors :-

- (1) Sialic acid
- (2) melanin
- (3) Urease
- (4) Superoxide dismutase
- (5) Mannitol fermentation
- (6) capsule
- (7) Mating Types

1° Infection \Rightarrow LUNGS \longrightarrow then Brain.

Δ - culture in Neger Seed Agar → Brown mucoid colonies
India ink.

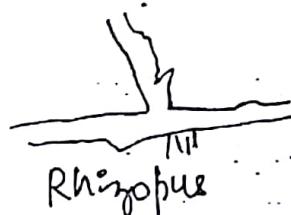
213

Ag Detection by Latex Agglutination.

IgA · IgG₂ → protective against capsule

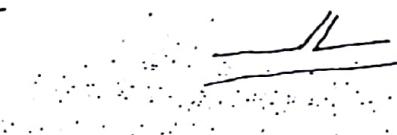
OBTUSE ZYgomycetes

Obtuse L



Nodat - Rhizoids

Mucor - No rhizoids



Absidia
Internodal rhizoids

Angio invasive → Rhinocerebral mucormycosis

↑ mortality

R/F - Diabetes ketoacidosis

Desferrioxamine Rx

Δ - Profuse Growth → Lid thrower.

ASEPTATE IRREGULAR BROAD, Ribbon like hyphae

Branching at OBTUSE ANGLE.

ASPERGILLUS

Fumigatus → Invasive (+ neutrophil)

Flavus → keratitis

Niger → Otomycosis

ACUTE ANGLE DICHOTOMOUS BRANCHING
'V' forms



PENICILLIUM MARNEFFI

umbilicated lesion. → like molluscum contagiosum
seen in HIV pt

A - Septate yeast: → Binary Fission

Red Pigments



PNEUMOCYSTIS JIROVECII

cause Interstitial Pneumonia (Non productive cough)

AIDS - induced sputum

HIV - BAL

A - can't be cultured

Gomori methamine silver → cyst wall

Toluidine Blue →

Giemsa → sporozoites (8 in No.)

Best DFA → Best Technique

Fungal Infection In Immunocompetent

Dermatophytes (MIC)

↓
Keratinophilic

Trichophyton (skin, hair, nail)	Macroconidia pencil shaped few	Microconidia plenty
------------------------------------	--------------------------------------	------------------------

215

Micromonascus (Skin, hair)	spindle (boat) plenty	few
-------------------------------	--------------------------	-----

Epidermophyton (skin, nail)	Clavate (club)	No
--------------------------------	----------------	----

Mentagrophytes → Hair Perforation (+)
(Trichophyton) Urine (-)

T. VERSICOLOR

— Malassezia globosa (M/e) → fuzzy —
— Lipophilic

Δ culture — SDA = olive oil
Spaghetti x Meat ball

TPN Rx → invasive-
(= lipids)

SPOROTHRRIX SCHENCKII

Thorn pick 'Rose Gardner's Disease'
Lymphatic spread → series of ulcer

Δ - Cigar shaped yeast
Rosette like conidia in SDA
'Splendore Hoepplii phenomenon'
(asteroid)
→ Cutaneous sycomycete
→ Blastomycosis

CHROMOBLASTOMYCOSIS

Coloured fungi (Pigmented) 216

A - **Sclerotic Body** → Brown septate Yeast appearing like "copper coin".

'BOOMERANG CONIDIA' CURVULARIA

Dermatophytes

- 1) Curvularia
- 2) Alternaria
- 3) Bipolaris
- 4) Cladophialophora
- 5) Exophiala
- 6)

- 6) Fonsecaea
- 7) Madurella
- 8) Scedosporium
- 9) Syzygopodium
- 10) Wangiella etc.

C/F - Verrucose cauliflower like lesion.

MYCETOMA

C/F

- Swelling
- Sinus
- Granuloma

RHINOSPORIDIUM SEEBERI

- PROTOZOA.
- Polyps. R/F → Pond Bathing
- A - 10% KOH → **spherules in endoscope**

SYSTEMIC FUNGI

Infective form \Rightarrow Arthroconidiae \rightarrow Spores in Hyphae 217

Pulmonary Lesions
TB like

A - Blastomycosis \rightarrow Broad Based budding

Paracoccidioidomycosis \rightarrow multiple budding yeast

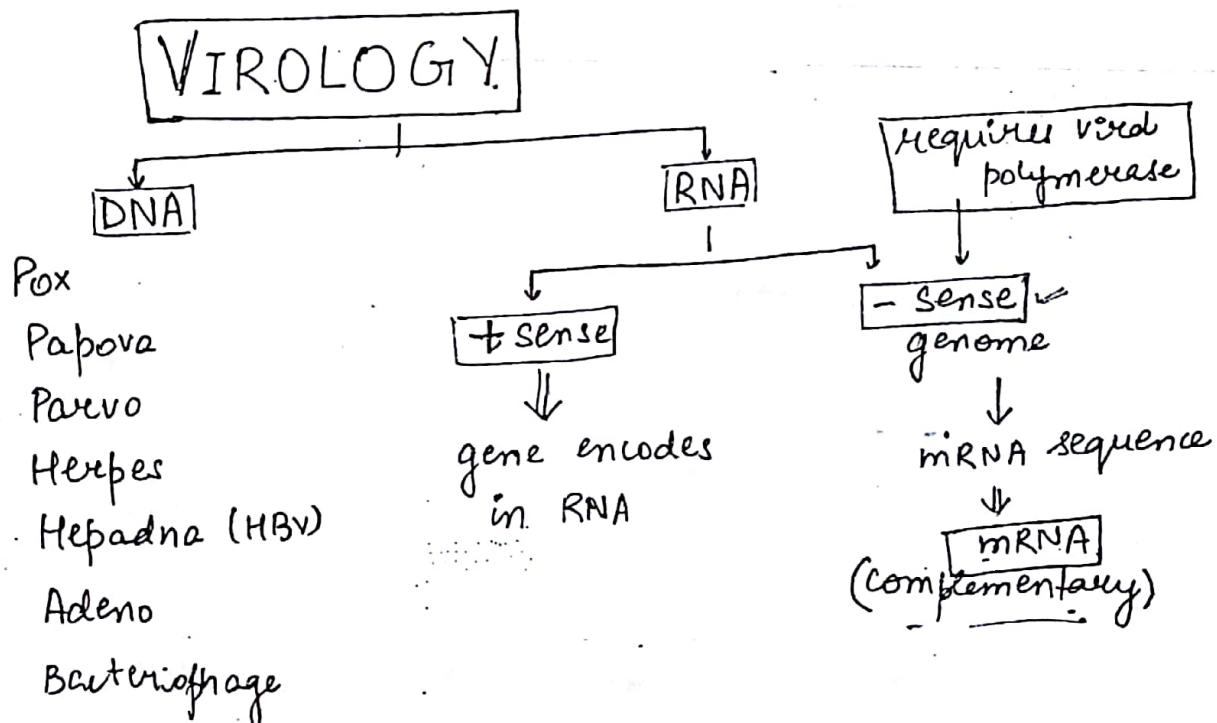
Martinez-Pelot wheel

Coccidioidomycosis \rightarrow Barrel shaped arthroconidia
Valley Fever

Desert Rheumatism

Spherules (tissue)

Histoplasma capsulatum
found in Bats \rightarrow Tubercular like projections
on 'spores'.



Non-Enveloped Virus

→ released by Lysis

218

DNA

Parvo

Adeno

Papova

RNA

Astro

Picorna

Reo

Calci

HAV

HEV

SP Cancel. H. RAE

Enveloped virus released by budding.

Segmented RNA viruses

Genetic re-assortment occurs.

They can show genetic shift.

B - Bunya virus (3 segments)

I - Influenza virus (8 segments)

R - Rota virus (11 segments)

A - Arenavirus (2 segments)

DNA VIRUSES

PARVO B19

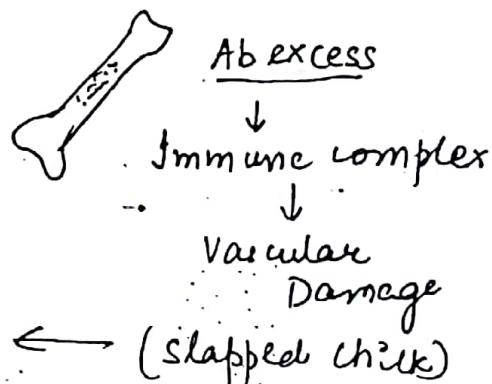
smallest 20 nm

affinity to immature RBC

immuno competent child.

(SMDs)

Erythema
Infectosum



Immuno competent Adult → Ab excess → polyarthropathy

Sickle cell → Ab response → Aplastic (Transient) crisis

Immuno compromised - No Ab response → Pure Red cell Aplasia (PRCA)

219

♀ → fetal liver, spleen, kidney ⇒ Hydrops Fetalis.

A - Ab detection

qPCR

Quantitative PCR (Real Time PCR) Best

↓
Taq man assay.

PAPOVA

POLIOMA = JC Virus → Progressive Multifocal leuko-
encephalopathy (PML)

BK virus → Kidney "Infer"

PAPILLOMA =

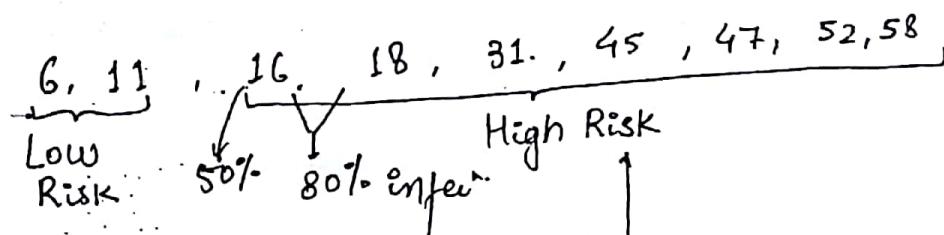
→ Warts

Ca Cx → **E protein** → Virulence

↓
Suppress the tumour

Suppressor genes

L protein → serotyping



VACCINE → Cervarix (16, 18)

Gardasil (6, 11, 16, 18)

Pzi Gardasil 9

Δ of Cx = Kolloidocyte → Cells = pyknotic nucleus
perinuclear halo

POX

Molluscum Contagiosum

220

↓
Pearly white umbilicated nodular Lesions

self limiting

direct contact

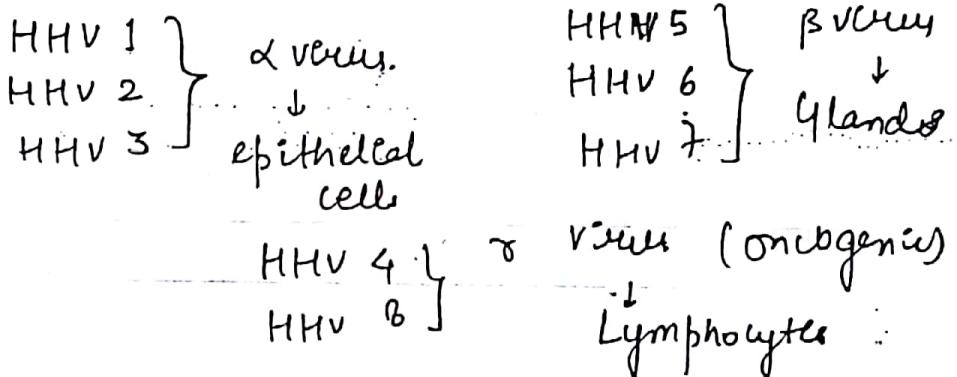
A - Molluscum Body (IB).

Bollinger Body → fowl pox [Inclusion Body
 Guineenese Body → vaccinia]

DNA virus → Intranuclear except Pox

RNA " → Intracytoplasmic except influenza, HIV

HERPES



HHV1

Stomatitis

Keratitis

Encephalitis

[Temporal → frontal]

less

HHV2

Genital infec'

Aseptic meningitis.

More virulent

Anti HSV → non protective

due to
+ trivial
exposure

1° Infection → Latency → Reactivation.

EPISOME (integration of viral DNA in host chromosome)

A- ~~darkroom~~ Tzanck Smear → Giemsa

221

Multinucleate Giant cell

Eosinophilic I.B (intrae~~n~~uclear)



Cowdry A

② Ab detection

③ PCR → IDC

HHV 3

- V-Z virus

Chickenpox → Lesions in crops.

Lifelong immunity in case of chickenpox

Shingles → Reactivation from Dorsal n/v root ganglion
of Trigeminal & Sacral n/v

↓
affect T₃ to L₂ U/L

Trigeminal, Facial
(Ophthalmic Div.)

Ramsay Hunt Syndrome.

Ant 2/3rd of Tongue

middle ear lesions

Belli's

Congenital V-Z Syndrome

Scarring of Leion (skin)

Hypoplasia of Limbs

Chorio retinitis

A- Tzanck

Ab detection

PCR

Vaccine = live strain.

222

Passive - VZ Ig.

HHV-4 → EBV

Reservoir → memory B cells

① Infection Mononucleosis → False + Serological Test
(Kissing Disease)

↓
Paul Bunnell Test (Heterophile agglutination test)
Sheep RBC

Differential Ab absorption Test

	Guinea Pig Kidney	OX RBC
Normal Serum Ab	adsorbed	(-)
Forsmann Ab (after Serum Rx)	adsorbed	adsorbed
IM	(-)	adsorbed

② Oral Hairy Leukoplakia

white plaque under Tongue. (self limiting)

children, HIV pt

↓
bleeds

③ Malignant - Nasopharyngeal Ca
Burkitt's Lymphoma
Hodgkin's.
NK/T cell.
B cell lymphoma

Lymphoproliferative Ds
Adenoma

HHV-5 CMV

enlargement of cells

223

Ubiquitous

Secreted in all body secretions



Retinitis in HIV + < 50 CD4

Inclusion Body Ds - children.

Transplantation

① Owl eye I.B.

② Ag Detect

③ PCR

HHV-6 - 6th Ds - ROSEOLA INFANTUM

or

Exanthem Subitum

HH7 → ↓ CD4 in HIV

HH8 → Kaposi's Sarcoma

ADENO VIRUS



Apical Fibres

"Space objects"

Serotype

8, 19, 37

→ Epidemic Keratoconjunctivitis

11, 21

→ Acute haemorrhagic cystitis

40, 41

→ Infantile Gastroenteritis

1, 2, 3, 5

→ Respiratory Infections

Vaccine: Live Non-attenuated Vaccine

Administered through Oral Route

Δ - ① Ab detection

② Culture in cell lines (human origin) 224

③

HELA/ HEP

Calx Ca Larynx

Balophilic I.B. [Cowdry B]
(intranuclear)

RNA VIRUSES

Infantile Gastroenteritis.

Rota Virus M/c → ds RNA

vomiting followed by diarrhea

family - Rota Reo

11 segments.

Vaccine - VPG + VP7

Intususception.

Δ - VPG Ag detection by ELISA in stool

Noro (Norwalk) → M/c in adults + children

CALCI

Sapo

ASTRO

Toga

Adeno-Type 40, 41 - DNA Virus (only)

Δ - can't be cultured

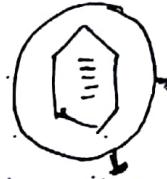
EM.

ORTHOFLUOXO

Influenza

Type A } 8 segments
B } HUMAN
C → 7 segments

Based on nucleocapsid protein.



H haemagglutinin 18 } Envelope
N neuraminidase 9 } Glycoprotein.

A - RT PCR.
Sample - nasopharyngeal swab.

AVIAN INFUENZA

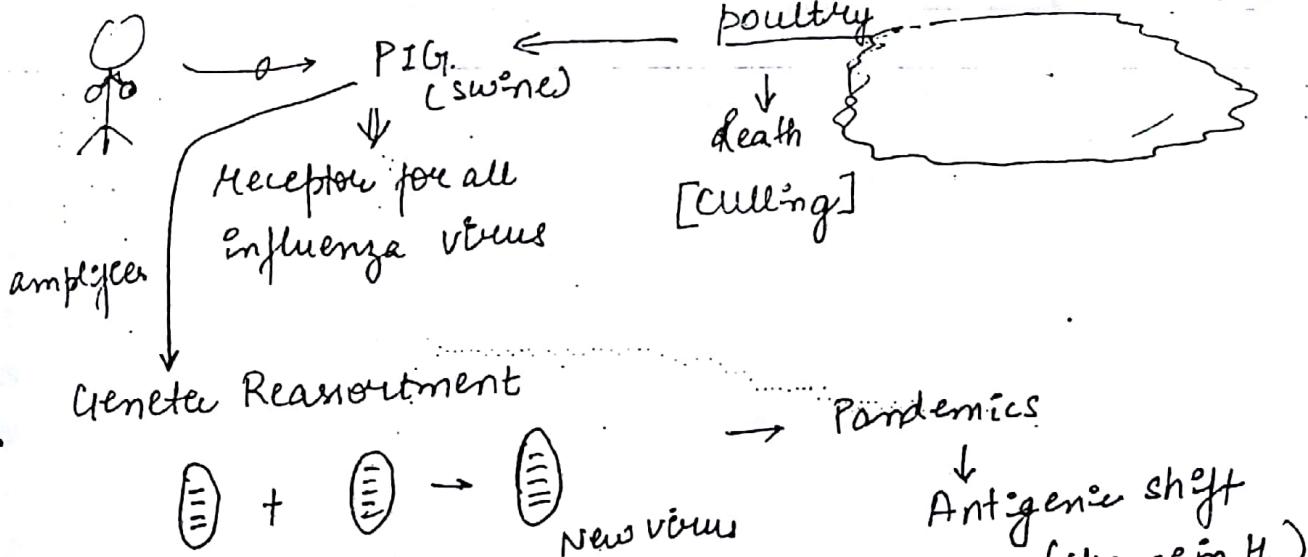
Reservoir $H_5 N_1$

↑ virulent

No person to person transmission.



Migratory Birds
(Reservoir)



Mutation → epidemics → antigenic drift.

$H_1 N_1 \rightarrow 1918$ Spanish flu

$H_2 N_2 \rightarrow 1957$ Asian

$H_3 N_2 \rightarrow 1968$ Hong Kong

Pig → Reservoir.

Von Magnus phenomenon

↑ titer (haemagglutinin)

↓ infectivity

Δ - RT PCR.

PARAMYXO

RSV → Bronchiolitis

Mumps → Parotitis - acute meningitis M/C
orchitis

Vaccine - Jeryl Lynn strain.

Measles → Fever & Rash (IP - 14 days)

Behind ears (4t)

KOPLIK'S SPOT → Opp. 2nd Molar (lower)

M/C complication - otitis media

M/C CNS " - Post measles Encephalitis
(autoimmune)
in 1 year.

SSPE - rare complication

5-25 years

due to defective virus. (spongiform
encephalitis)

Prion proteins

Δ - Ab detection (after 7 days of onset of rash)

PCR (nasopharyngeal swab)

in 5 days.

RUBELLA

German Measles

Vaccine → RA 27/3

Adults → Exanthematous Rash / Keratitis.

Congenital Rubella Syndrome

Cataract

Deafness

Heart Defects

Δ - Ab detection

⑥ ♀ 1st Trimester - is exposed to her friend suspected of Rubella - What next?

susceptibility



♀ → diagnose

If friend have IgM +ve

a) Ig G

then check for IgG in ♀

b) Ig G, Ig M

Ig G +ve → no worry

c) Ig G

Ig G -ve → come for next month

Ig M

Ig M +ve → abortion.

d) -

Ig M

Ig M.

ARBOVIRUS

arthropod virus

→ mosquito + Ticks.

Flav: Virus

Bunya "

Toga "

Ticks

228

✓ Powassan

✓ Russian spring Summer

✓ Kyasanur forest disease

✓ CHF (Crimean Congo HF) haemorrhagic fever

DENGUE

5 serotypes

DF → DHF / DSS

Ab enhancement

Immune complex

* Factors affecting Haemorrhage :-

1) Repeat infection in different serotype

2) Sequence of infection. Type 1 followed by Type 2

3) Serotype 2

4) whites

5) < 12 yrs.

6) Female

→ Malnutrition → protective

Δ - IgM Capture ELISA

NS1 Ag (in 5 days)

ZIKA

* Microcephaly in newborn.

JE → pig → amplifying host

Δ - Ab detection

Vaccine - SA - 14-14-2 (Live) : 1 dose
↓
tissue culture

229

CCHF (Crimmenn Congo Haemorrhagic Fever)
Case reported in Gujarat, Rajasthan.

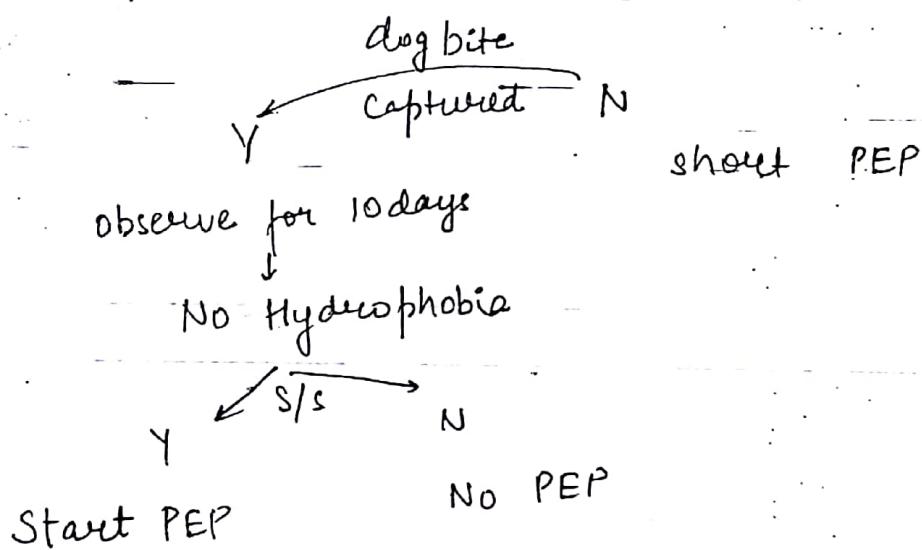
A - Ab detection

PCR

Rhabdovirus

Bullet shaped

3mm/hr or 230 mm/day [Rate of spread].



Postmortem (behead dog) → Brain.

↓
Sellar stalk

↓
Negri Body → + Hippocampus
cerebellum

POLIO

230

AFP

Δ - stool culture (2 samples)

↓
Sequencing → Type 1 or 2 or VDPP

HEPATITIS

HAV (Picornavirus)

feco-oral route → acute infect

↓
fulminancy in adults
outbreak

Δ - only hepatitis virus cultured in cell line

IgM Anti-HAV

↓
one serotype & 4 genotype

HEV (Hepe virus)

feco-oral route → acute infect

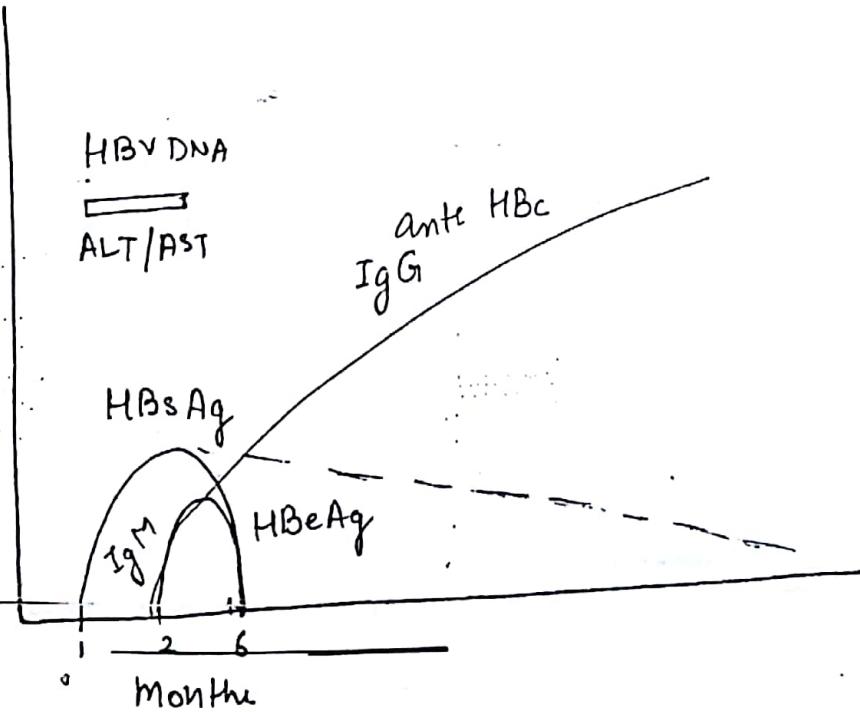
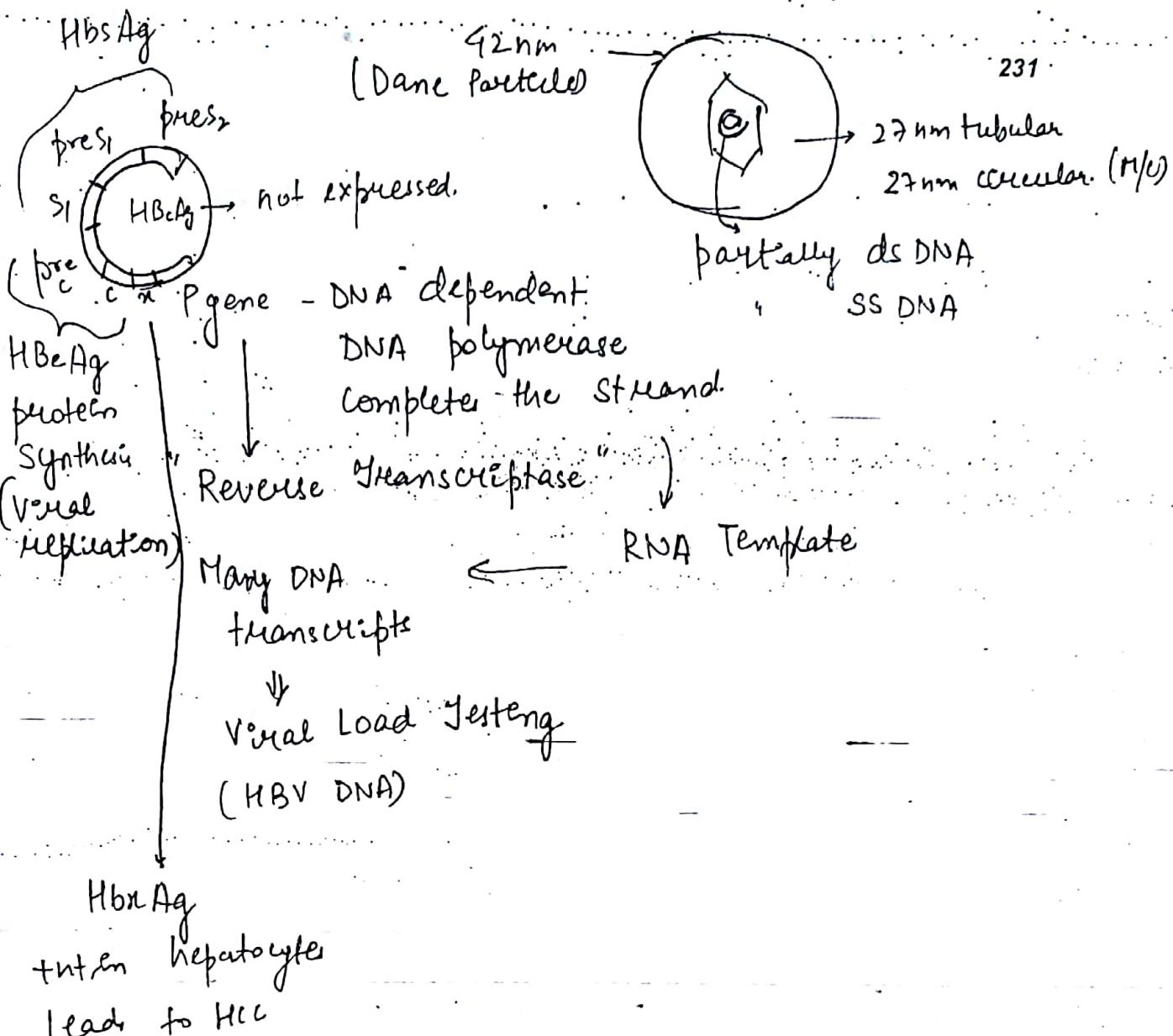
↓
fulminant in pregnancy

outbreak

Blood Transmission (Rarely documented)

Δ - IgM anti HEV

HBV (Hepadnavirus)



Acute Hep B infec' in adult \rightarrow usually resolves 232

Vertical transmission \rightarrow chronicity ↑

(M/c)

Best Marker of Acute Hep B \Rightarrow IgM Anti HBc

Chronic active \rightarrow Infections Require Rx
supercarrier



IgG anti-HBc + HBV DNA

Chronic persistent \rightarrow IgG anti-HBc (+) HBV DNA (-)

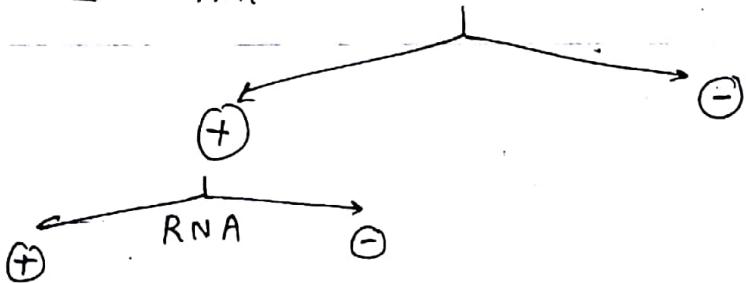
HCV flavivirus.

Chronic infec' ↑↑

acute \rightarrow No signs / symptoms.

unsafe inject' \rightarrow (M/c)

Δ - Anti HCV



Rx

Sofosbuvir +
daclatasvir.

3 months

genotyping not Req

Ribavirin + IFN \rightarrow genotyping

Indea Type 3 > 1

HDV

Co-infer → IgM Anti HBc

233

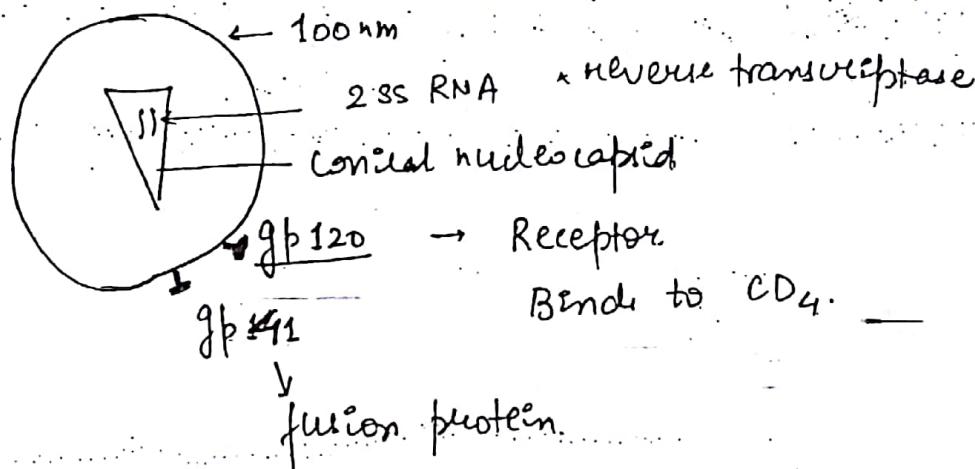
Super infer → IgG anti HBc

fulminant

↑ incidence of fulminancy - 20%

H/c fulminancy → HBV

HIV



Co-receptor on the host cell

(CCR5)

CXCR4

(R₅ virus)

(X₄ virus)

↓

M trophic

↓

T trophic

Monocytes/
macrophages

Lymphocyte

↓
Resistance

HIV 1
 M N O
 Major Human

HIV 2 234
 West Africa
 Intense (P) to NNRTI

Subtype - C → India

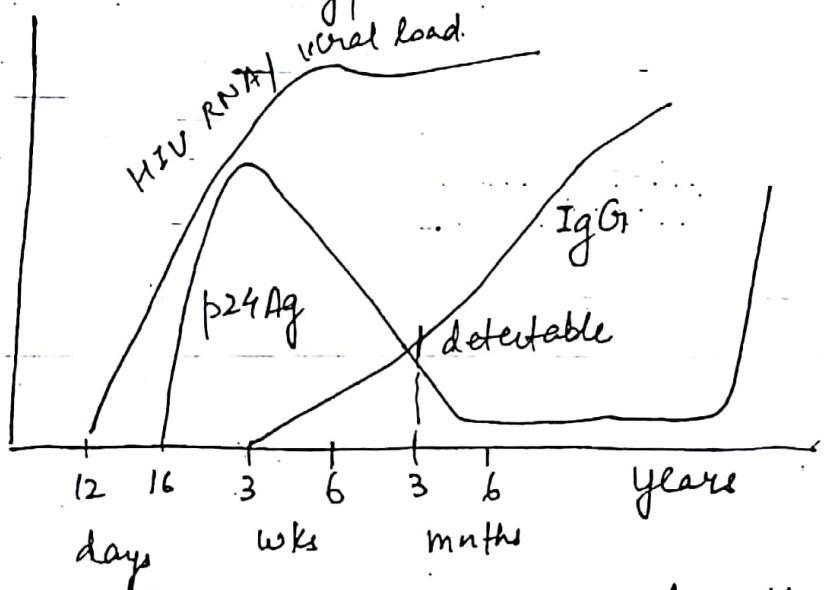
Env gene is concerned between HIV 1 + 2

(gag-pol share 90% homology)

env Ag used for diagnosis

gp 120 & gp 41 → HIV 1

gp 36 → HIV 2



Recent H/o exposure p24Ag → day 16 to 3 months

Δ of HIV → Ab detection

Most sensitive → 6th generation
 ELISA

detects both p24 Ag, HIV Ab
 3 tests

Confirmation → western Blot

A. in children - [DNA PCR] Q.

235

"dried blood spot"

Disease Monitoring

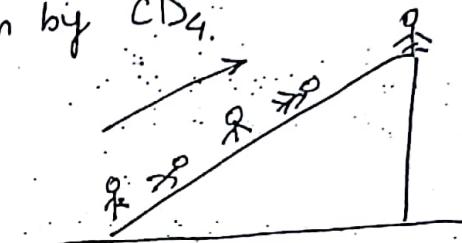
CD₄ → opportunistic inf., treatment

Viral Load → Prognosis

Present immune status given by CD₄.

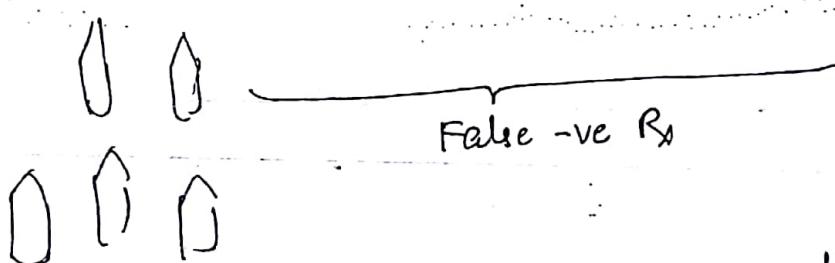
Rapid progressor (sympt.)

Slow "



SEROLOGY

Ag - Ab Rxn.



If Ab excess \Rightarrow PROZONE

If Ag excess.
POST ZONE



zone of equivalence

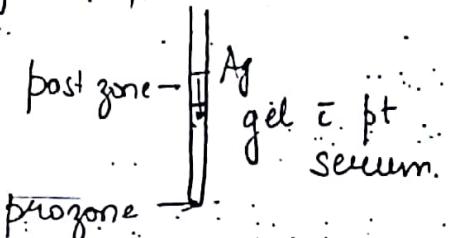
Serological Test \rightarrow dilution (2-fold serial)
of serum

Titre \rightarrow Highest Dilution at \pm Rxn is seen.

Soluble Ag binds to Ab

Ab to form soluble ppt

Single diffusion in one dimension Oudin procedure



Double diffusion in one dimension Oukley Fulthrop procedure

Single diffusion in two dimensions Radial Immuno diffusion

Double-diffusion in two dimensions Ouchterlony procedure

e.g. Elek gel's electrophoresis

Quantitative → Rocket Electrophoresis

Agglutination

Particulate Ag binds to Ab to form visible clumps



Passive Agglutination → Particles to detect Ab

Reverse " → particle to detect Ag

Complement Fixation

237.

Detects Ab.

ELISA is used nowadays \rightarrow as it detects Ag + Ab

ELISA large no. of samples ~~can~~ tested at 1 time

Direct ELISA

detects Ag

specific

Indirect ELISA

detects Ab

sensitive

Competitive ELISA

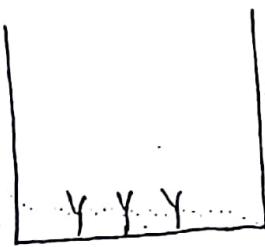
detects Ab \rightarrow highly specific

Capture ELISA

detects isotype of Ab \Rightarrow

using monoclonal Ab \rightarrow isotypes are captured i.e.

e.g. Dengue IgM.



mAb for IgM.

add Dengue Ag + Ab + antigen

↓ now add substrate
+ enzyme

colour change

Immunofluorescence

238

FITC dye

fluorescein isothiocyanate.

Chemiluminescence (CLIA)

→ light emitting particles

10 - 100 times more sensitive than ELISA.

Obligate Intracellular Parasite

CRV CM PTI

Chlamydia

Mycobacterium leprae

Rickettsia (Ehrlichia, Anaplasma)

Plasmodium sp.

Venereal

Pneumocystis jirovecii

Coxiella Burnetii

Toxoplasma gondii

Cryptosporidium parvum

Typhpanoma cyste

Leishmania Donovani

Facultative Intracellular Parasite

MBBS CRY for NH2

Mycobacterium

Francisella tularensis

Bartonella henselae

Nocardia

Bacillus

N. meningitidis

Salmonella Typhi

Helicobacter capsulatum

Cryptococcus neoformans

Listeria monocytogene

Rhodococcus equi

Legionella

Yersinia